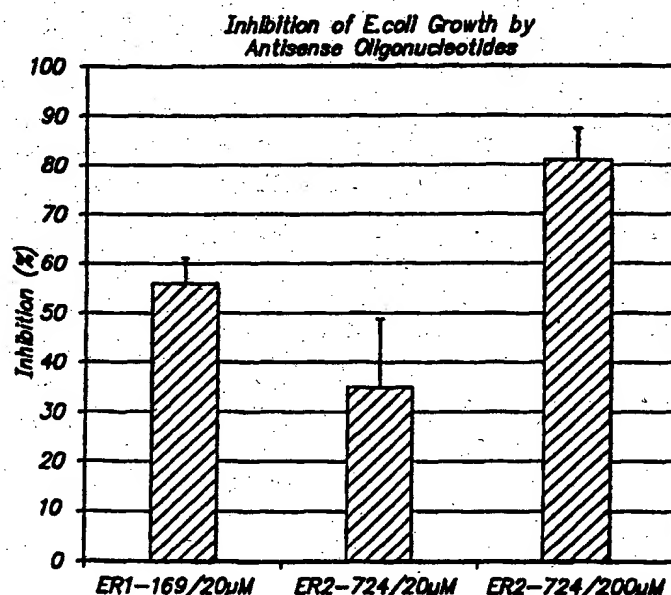




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(54) Title: **ANTISENSE OLIGONUCLEOTIDE SEQUENCES AS INHIBITORS OF MICROORGANISMS**

(57) Abstract

The invention relates to antisense oligonucleotides which modulate the expression of the ribonucleotide reductase or the *secA* genes in microorganisms. This invention is also related to methods of using such oligonucleotides in inhibiting the growth of microorganisms. These antisense oligonucleotides are particularly useful in treating pathological conditions in mammals which are mediated by the growth of microorganisms.

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ANTISENSE OLIGONUCLEOTIDE SEQUENCES AS INHIBITORS OF MICROORGANISMS

BACKGROUND OF THE INVENTION

Field of the Invention

This invention relates to antisense oligonucleotides which modulate the activity of the ribonucleotide reductase genes and the secA genes in microorganisms. This invention is also related to methods of using such compounds in inhibiting the growth of microorganisms.

These antisense oligonucleotides are particularly useful in treating pathological conditions in mammals which are mediated by the growth of microorganisms.

Accordingly, this invention also relates to pharmaceutical compositions comprising a pharmaceutically acceptable excipient and an effective amount of a compound of this invention.

These antisense oligonucleotides may also be used as anti-microbial agents for agricultural applications such as crop protection.

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All of the above publications, patent applications and patents are herein incorporated by reference in their entirety to the same extent as if each individual
5 publication, patent application or patent was specifically and individually indicated to be incorporated by reference in its entirety.

State of the Art

Ribonucleotide reductase catalyzes the *de novo* production of
10 deoxyribonucleotides. The enzyme reduces the four main ribonucleotides to the corresponding deoxyribonucleotides required for DNA synthesis and repair (Wright et al.⁴¹).

In mammalian and bacterial cells, *de novo* production of deoxyribonucleotides by ribonucleotide reductase is usually highly regulated on different levels in order to
15 produce the correct amount of deoxyribonucleotides for DNA synthesis. In the DNA viruses, the metabolism of the host cell is directed towards production of viral DNA by virus encoded ribonucleotide reductases (Nordlund and Eklund¹).

Mammalian cells and many DNA viruses and prokaryotes, have a heterodimeric iron-containing ribonucleotide reductase enzyme of the $\alpha_2\beta_2$ type. For
20 example, ribonucleotide reductase from *E. coli* is a multi-subunit $\alpha_2\beta_2$ enzyme where the two homo-dimeric proteins are denoted R1 and R2. The larger α_2 protein, R1, contains the binding sites for substrate and allosteric effectors and also the redox-active cysteine residues. Protein R1 has a molecular mass of 2 x 86,000 where each subunit contains 761 residues. The smaller β_2 protein, denoted R2, contains the dinuclear
25 ferric center and a stable free tyrosyl radical necessary for the enzymatic activity. The R2 protein has a molecular mass of 2 x 43,500, where each subunit contains 375 amino acid residues (Nordlund and Eklund¹).

The nucleotide sequence of the *E. coli* K-12 DNA comprising the operon for the structural genes of the subunits of ribonucleotide reductase has been determined. The
30 DNA sequence includes a total length of 8557 nucleotides. An open reading frame

between nucleotides 3506 and 5834 has been identified as the *nrdA* gene. An open reading frame between nucleotides 6012 and 7139 encoding a 375-amino acid polypeptide has been identified as the *nrdB* gene (Carlson et al.², and Nilsson et al.³). The sequences of the *nrdA* and *nrdB* genes for *E. coli* are shown in Figures 1 and 2.

5 In *E. coli*, the synthesis of ribonucleotide reductase is controlled at the level of transcription. The *nrdA* and *nrdB* genes direct the synthesis of a 3.2 kilobase polycistronic mRNA. Perturbations in DNA replication, either a shift up in growth conditions or an inhibition of DNA synthesis leads to increased synthesis of *nrd* mRNA (Carlson et al.²).

10 A separate anaerobic ribonucleotide reductase has also been identified from *E. coli*. The anaerobic *E. coli* reductase has a molecular mass of 145 kD and is a homodimer. The gene for the anaerobic reductase (*nrdD*) has been cloned and sequenced (P. Reichard⁴).

15 The ribonucleotide reductase R2 genomic or cDNA sequences are known for several other species such as bacteriophage T4, clam, mouse, *Saccharomyces cerevisiae*, vaccinia, herpes simplex virus types 1 and 2, varicella and Epstein-Barr virus (Nordlund et al.⁵). The sequence of the *nrdE* and *nrdF* which code for the ribonucleotide reductase genes of *S. typhimurium* are shown in Figure 3. The sequence of the ribonucleotide reductase gene of *Lactococcus lactis* is shown in Figure 4.

20 The *secA* gene of *E. coli* encodes for one component of a multi-component system for the secretion of proteins across the inner membrane of *E. coli* (der Blaauwen et al.⁶). The complete system consists of the SecB protein, a cytosolic chaperone, the SecA protein, the translocation ATPase and the heterotrimeric integral membrane SecY/SecE/SecG complex, which along with SecA serves as the preprotein channel. SecA protein plays a central role in the secretion process by binding the
25 preprotein, secB protein, anionic phospholipids and SecY/SecE/SecG protein. These interactions allow SecA to recognize soluble preprotein and recruit it to translocation sites in the inner membrane. Once such protein translocation complexes have assembled; further steps require an ATP-driven cycle of insertion and de-insertion of

secA protein with the inner membrane, where each cycle appears to be coupled to the translocation of a segment of the preprotein.

SecA is the only component of the secretion apparatus that has been shown to be regulated. SecA is the second gene in the geneX-secA operon and its translation varies
5 over a tenfold range depending on the status of protein secretion in the cell. During protein-export proficient conditions secA auto-represses its translation by binding to a site that overlaps the secA ribosome-binding site of genes-secA RNA. SecA protein can also dissociate a preformed 30 S-tRNA^{MET}-genes-secA RNA ternary complex in vitro. However, during a protein export block secA translation increases substantially
10 although the mechanism responsible for this regulatory response has not been elucidated (McNicholas et al.⁷). The sequence of the secA gene of *E. coli* is shown in Figure 5.

The secA gene sequence has been identified for a number of other species including *Mycobacterium bovis* (Figure 6), *Mycobacterium tuberculosis* (Figure 7),
15 *Staphylococcus aureus* (Figure 8), *Staphylococcus carnosus* (Figure 9), *Bacillus subtilis*, *Bacillus firmus*, *Listeria monocytogenes*, *Mycobacterium smegmatis*, *Borrelia burgdorferi*, *P. sativum*, *S. griseus*, and *Synechococcus sp.*

Antibiotics are important pharmaceuticals for the treatment of infectious diseases in a variety of animals including man. The tremendous utility and efficacy of
20 antibiotics results from the interruption of bacterial (prokaryotic) cell growth with minimal damage or side effects to the eukaryotic host harboring the pathogenic organisms. In general, antibiotics destroy bacteria by interfering with the DNA replication, DNA to RNA transcription, translation (that is RNA to protein) or cell wall synthesis.

25 Although bacterial antibiotic resistance has been recognized since the advent of antimicrobial agents, the consequence of the emergence of resistant microorganisms, such resistance was historically controlled by the continued availability of effective alternative drugs. Now, drug resistance has emerged as a serious medical problem in the community, leading to increasing morbidity and mortality. The problem is
30 worsened by the growing number of pathogens resistant to multiple, structurally

unrelated drugs. The situation has become so desperate that antibiotics once removed from use because of toxic effects may be prescribed in an attempt to deal with the otherwise untreatable drug resistant bacteria.

Antisense oligonucleotides have been used to decrease the expression of specific genes by inhibiting transcription or translation of the desired gene and thereby achieving a phenotypic effect based upon the expression of that gene (Wright and Anazado³⁸). For example, antisense RNA is important in plasmid DNA copy number control, in development of bacteriophage P22. Antisense RNA's have been used experimentally to specifically inhibit *in vitro* translation of mRNA coding specifically from *Drosophila hsp23*, to inhibit Rous sarcoma virus replication and to inhibit 3T3 cell proliferation when directed toward the oncogene *c-fos*. Furthermore, it is not necessary to use the entire antisense mRNA since a short antisense oligonucleotide can inhibit gene expression. This is seen in the inhibition of chloramphenicol acetyltransferase gene expression and in the inhibition of specific antiviral activity to vesicular stomatitis virus by inhibiting the N-protein initiation site. Antisense oligonucleotides directed to the macromolecular synthesis operon of bacteria, containing the *rpsU* gene, the *rpoD* gene and the *dnaG* gene have been used for the detection of bacteria. (U.S. Patent No. 5,294,533⁸). Furthermore, photoactivatable antisense DNA complementary to a segment of the β -lactamase gene has been used to suppress ampicillin resistance in normally resistant *E. coli* (Gasparro et al.⁹). Antisense DNA analogs have also been used to inhibit the multiple antibiotic resistant (*mar*) operon in *Escherichia coli* (White et al.¹⁰).

Accordingly, there is a need to develop antisense oligonucleotides which will act to inhibit the growth of microorganisms.

25

SUMMARY OF THE INVENTION

This invention is directed to antisense oligonucleotides which modulate the expression of the ribonucleotide reductase and *secA* genes in microorganisms and pharmaceutical compositions comprising such antisense oligonucleotides. This

invention is also related to methods of using such antisense oligonucleotides for inhibiting the growth of microorganisms.

Accordingly, in one of its composition aspects, this invention is directed to an antisense oligonucleotide, which oligonucleotide is nuclease resistant and comprises
5 from about 3 to about 50 nucleotides, which nucleotides are complementary to the ribonucleotide reductase gene or the secA gene of a microorganism. The antisense oligonucleotide may have one or more phosphorothioate internucleotide linkages.

In another of its composition aspects, this invention is directed to an antisense oligonucleotide comprising from about 3 to about 50 nucleotides which is capable of
10 binding to the ribonucleotide reductase gene or the secA gene of a microorganism, wherein the oligonucleotide comprises all or part of a sequence selected from the group consisting of SEQ ID NO:22; SEQ ID NO:43; SEQ ID NO:62; SEQ ID NO:74; SEQ ID NO:75; SEQ ID NO:76; SEQ ID NO:143; SEQ ID NO:145; SEQ ID NO:152; SEQ ID NO:164; SEQ ID NO:176; SEQ ID NO:186; SEQ ID NO:188; SEQ ID
15 NO:189; SEQ ID NO:191; SEQ ID NO:192; SEQ ID NO:195; SEQ ID NO:197; SEQ ID NO:206; SEQ ID NO:212; SEQ ID NO:220; SEQ ID NO:229; SEQ ID NO:235; SEQ ID NO:254; SEQ ID NO:261; SEQ ID NO:262; SEQ ID NO:263; SEQ ID NO:264; and SEQ ID NO:265.

In still another of its composition aspects, this invention is directed to a
20 pharmaceutical composition comprising a pharmaceutically acceptable excipient and an effective amount of an antisense oligonucleotide, which oligonucleotide is nuclease resistant and comprises from about 3 to about 50 nucleotides, which nucleotides are complementary to the ribonucleotide reductase gene or the secA gene of a microorganism. The oligonucleotide may be modified, for example, the
25 oligonucleotide may have one or more phosphorothioate internucleotide linkages.

In one of its method aspects, this invention is directed to a method for inhibiting the expression of the ribonucleotide reductase gene in a microorganism having a ribonucleotide reductase gene comprising, administering to said microorganism or to a cell infected with said microorganism an effective amount of an antisense
30 oligonucleotide comprising from at least about 3 nucleotides which are complementary

to the ribonucleotide reductase gene of the microorganism under conditions such that the expression of the ribonucleotide reductase gene is inhibited.

In another of its method aspects, this invention is directed to a method for inhibiting the expression of the secA gene in a microorganism having a secA gene, comprising administering to said microorganism an effective amount of an antisense oligonucleotide comprising from at least about 3 nucleotides which are complementary to the secA gene of the microorganism under conditions such that expression of the secA gene is inhibited.

In one of its method aspects, this invention is directed to a method for inhibiting the growth of a microorganism encoding a ribonucleotide reductase gene or a secA gene, which method comprises administering to said microorganism or a cell infected with said microorganism an effective amount of an antisense oligonucleotide comprising from at least about 3 nucleotides which are complementary to either the ribonucleotide reductase gene or the secA gene of the microorganism under conditions such that the growth of the microorganism is inhibited. Preferably, the antisense oligonucleotide is selected from the group consisting of SEQ ID NO:22; SEQ ID NO:43; SEQ ID NO:62; SEQ ID NO:74; SEQ ID NO:75; SEQ ID NO:76; SEQ ID NO:143; SEQ ID NO:145; SEQ ID NO:152; SEQ ID NO:164; SEQ ID NO:176; SEQ ID NO:186; SEQ ID NO:188; SEQ ID NO:189; SEQ ID NO:191; SEQ ID NO:192; SEQ ID NO:195; SEQ ID NO:197; SEQ ID NO:206; SEQ ID NO:212; SEQ ID NO:220; SEQ ID NO:229; SEQ ID NO:235; SEQ ID NO:254; SEQ ID NO:261; SEQ ID NO:262; SEQ ID NO:263; SEQ ID NO:264; and SEQ ID NO:265.

In another of its method aspects, this invention is directed to a method for treating a mammalian pathologic condition mediated by a microorganism, which method comprises identifying a mammal having a pathologic condition mediated by a microorganism having a ribonucleotide reductase gene or a secA gene and administering to said mammal an effective amount of an antisense oligonucleotide comprising from at least about 3 nucleotides which are complementary to either the ribonucleotide reductase gene or the secA gene of the microorganism under conditions such that the growth of the microorganism is inhibited.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is the sequence of the *E. coli* nrdA gene encoding the ribonucleotide reductase R1 subunit [SEQ ID NO:1].

Figure 2 is the sequence of the *E. coli* nrdB gene encoding the ribonucleotide reductase R2 subunit [SEQ ID NO:2]. The nrdB gene is encoded by nucleotides 7668 to 8798 of SEQ ID NO:2.

Figure 3 is the sequence of the *S. typhimurium* nrdE and nrdF genes encoding the ribonucleotide reductase subunits [SEQ ID NO:3]. The nrdE gene is encoded by nucleotides 836 to 2980 and the nrdF gene is encoded by nucleotides 2991 to 3950 of SEQ ID NO:3.

Figure 4 is the sequence of the *Lactococcus lactis* nrdEF operon encoding ribonucleotide reductase [SEQ ID NO:4].

Figure 5 is the sequence of the *E. coli* secA gene [SEQ ID NO:5].

Figure 6 is the sequence of the *Mycobacterium bovis* secA gene [SEQ ID NO:6].

Figure 7 is the sequence of the *Mycobacterium tuberculosis* secA gene [SEQ ID NO:7].

Figure 8 is the sequence of the *Staphylococcus aureus* secA gene [SEQ ID NO:8].

Figure 9 is the sequence of the *Staphylococcus carnosus* secA gene [SEQ ID NO:9].

Figure 10 is the sequence of the bovine herpes virus ribonucleotide reductase small subunit gene [SEQ ID NO:10].

Figure 11 is the sequence of the Herpes simplex virus type 1 UL39 gene encoding ribonucleotide reductase 1 [SEQ ID NO:11].

Figure 12 is the sequence of the Herpes simplex type 2 ribonucleotide reductase gene [SEQ ID NO:12]. The ribonucleotide reductase gene is encoded by nucleotides 419 to 3853 of SEQ ID NO:12.

Figure 13 is the sequence of the equine herpes virus 4 ribonucleotide reductase large subunit and small subunit [SEQ ID NO:13]. The large subunit is encoded by

nucleotides 77 to 2446 and the small subunit by nucleotides 2485-3447 of SEQ ID NO:13.

Figure 14 is a photograph of a Western blot of a polyacrylamide gel of the cellular protein from *E. coli* cells carrying a plasmid containing the mouse ribonucleotide reductase R2 gene after treatment with either 20 μ M or 200 μ M of oligonucleotide AS-II-626-20.

Figure 15 is a graph of the inhibition of *E. coli* growth after treatment of *E. coli* cells with ribonucleotide reductase antisense oligonucleotides.

Figure 16 is a graph of the number of colony forming units/ml of *E. coli* cells after treatment with ribonucleotide reductase antisense oligonucleotides.

Figure 17 is a photograph of a Western blot of a polyacrylamide gel of cellular protein from *E. coli* cells after treatment with secA antisense oligonucleotides.

Figures 18a and 18b are graphs of the number of colony forming units/ml of *E. coli* cells after treatment with secA antisense oligonucleotides.

Figures 19a-g are graphs of growth curves of *E. coli* K12 after treatment with antisense oligonucleotides. Figure 19a shows the growth after treatment with 16 μ M or 80 μ M of antisense ES799 [SEQ ID NO:195]. Figure 19b shows the growth after treatment with 20 μ M of antisense ES1739 [SEQ ID NO:229]. Figure 19c shows the growth after treatment with 80 μ M of antisense ES851 [SEQ ID NO:197]. Figure 19d shows the growth after treatment with 80 μ M of antisense ES553 [SEQ ID NO:188]. Figure 19e shows the growth after treatment with 80 μ M of antisense ES646 [SEQ ID NO:191]. Figure 19f shows the growth after treatment with 80 μ M of antisense ES1845 [SEQ ID NO:235]. Figure 19g shows the growth after treatment with 80 μ M of antisense ES2537 [SEQ ID NO:254].

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides compounds that inhibit the growth of microbes by inhibiting the expression of a ribonucleotide reductase protein or the secA protein. Without being limited to any theory, the compounds inhibit the expression of the ribonucleotide reductase or the secA protein by inhibiting the transcription of the gene

or the translation of the mRNA to protein. Such compounds include antisense oligonucleotides.

Definitions:

5 As used herein, the following terms have the following meanings:

The term "antisense oligonucleotide" as used herein means a nucleotide sequence that is complementary to the mRNA for the desired gene. Preferably, the antisense oligonucleotide is complementary to the mRNA for ribonucleotide reductase or secA.

10 The term "oligonucleotide" refers to an oligomer or polymer of nucleotide or nucleoside monomers consisting of naturally occurring bases, sugars, and inter-sugar (backbone) linkages. The term also includes modified or substituted oligomers comprising non-naturally occurring monomers or portions thereof, which function similarly. Such modified or substituted oligomers may be preferred over naturally
15 occurring forms because of the properties such as enhanced cellular uptake, or increased stability in the presence of nucleases. The term also includes chimeric oligonucleotides which contain two or more chemically distinct regions. For example, chimeric oligonucleotides may contain at least one region of modified nucleotides that confer beneficial properties (e.g. increased nuclease resistance, increased uptake into
20 cells) or two or more oligonucleotides of the invention may be joined to form a chimeric oligonucleotide.

The antisense oligonucleotides of the present invention may be ribonucleic or deoxyribonucleic acids and may contain naturally occurring or synthetic monomeric bases, including adenine, guanine, cytosine, thymine and uracil. The oligonucleotides
25 may also contain modified bases such as xanthine, hypoxanthine, 2-aminoadenine, 6-methyl, 2-propyl and other alkyl adenines, 5-halo uracil, 5-halo cytosine, 6-aza uracil, 6-aza cytosine and 6-aza thymine, pseudo uracil, 4-thiouracil, 8-halo adenine, 8-aminoadenine, 8-thiol adenine, 8-thiolalkyl adenines, 8-hydroxyl adenine and other 8-substituted adenines, 8-halo guanines, 8-amino guanine, 8-thiol guanine, 8-thioalkyl
30 guanines, 8-hydroxyl guanine and other 8-substituted guanines, other aza and deaza

uracils, thymidines, cytosines or guanines, 5-trifluoromethyl uracil and 5-trifluoro cytosine.

The antisense oligonucleotides of the invention may also comprise modified phosphorus oxygen heteroatoms in the phosphate backbone, short chain alkyl or cycloalkyl intersugar linkages or short chain heteroatom or heterocyclic intersugar linkages. For example, the antisense oligonucleotides may contain methyl phosphonates, phosphorothioates, phosphorodithioates, phosphotriesters, and morpholino oligomers. In one embodiment of the invention, the antisense oligonucleotides comprise phosphorothioate bonds linking between the four to six 3'-terminus nucleotides. In another embodiment, the phosphorothioate bonds link all the nucleotides. The antisense oligonucleotides may also have sugar mimetics.

The antisense oligonucleotides of the invention may also comprise nucleotide analogues wherein the structure of the nucleotide is fundamentally altered. An example of such an oligonucleotide analogue is a peptide nucleic acid (PNA) wherein the deoxyribose (or ribose) phosphate backbone in DNA (or RNA) is replaced with a polyamide backbone which is similar to that found in peptides (Nielsen et al.¹¹; Good and Nielsen¹²; Buchardt, deceased, et al.¹³, U.S. Patent No. 5,766,855; Buchardt, deceased, et al.¹⁴, U.S. Patent No. 5,719,262). PNA analogues have been shown to be resistant to degradation by enzymes and to have extended lives *in vivo* and *in vitro*. PNAs also bind more strongly to a complementary DNA sequence than to a naturally occurring nucleic acid molecule due to the lack of charge repulsion between the PNA strand and the DNA strand.

The oligonucleotides of the present invention may also include other nucleotides comprising polymer backbones, cyclic backbones, or acyclic backbones. For example, the nucleotides may comprise morpholino backbone structures (U.S. Patent No. 5,034,506¹⁵).

The oligonucleotides of the present invention are "nuclease resistant" when they have either been modified such that they are not susceptible to degradation by DNA and RNA nucleases or alternatively they have been placed in a delivery vehicle which in itself protects the oligonucleotide from DNA or RNA nucleases. Nuclease resistant

oligonucleotides include, for example, methyl phosphonates, phosphorothioates, phosphorodithioates, phosphotriesters, and morpholino oligomers. Suitable delivery vehicles for conferring nuclease resistance include, for example liposomes.

5 The oligonucleotides of the present invention may also contain groups, such as groups for improving the pharmacokinetic properties of an oligonucleotides, or groups for improving the pharmacodynamic properties of an oligonucleotide. Preferably, the oligonucleotides do not contain reporter groups or labels, such as fluorescent dyes or radioactive labels.

10 The antisense oligonucleotides may be complementary to the complete ribonucleotide reductase or secA gene including the introns. Preferably, the antisense oligonucleotides are complimentary to the mRNA region from the ribonucleotide reductase gene or the secA gene.

15 The antisense oligonucleotides may be selected from the sequence complementary to the ribonucleotide reductase or secA genes such that the sequence exhibits the least likelihood of showing duplex formation, hair-pin formation, and homooligomer/sequence repeats but has a high to moderate potential to bind to the ribonucleotides reductase gene or the secA gene sequence and contains a GC clamp. These properties may be determined using the computer modeling program OLIGO Primer Analysis Software, Version 5.0 (distributed by National Biosciences, Inc.,
20 Plymouth, MN). This computer program allows the determination of a qualitative estimation of these five parameters.

Alternatively, the antisense oligonucleotides may also be selected on the basis that the sequence is highly conserved for either the ribonucleotide reductase or the secA genes between two or more microbial species. These properties may be determined
25 using the BLASTN program (Altschul, et al.¹⁶) of the University of Wisconsin Computer group (GCG) software (Devereux J. et al.¹⁷) with the National Center for Biotechnology Information (NCBI) databases.

The antisense oligonucleotides generally comprise from at least about 3 nucleotides or nucleotide analogs, preferably from about 3 to about 50 nucleotides or

nucleotide analogs, more preferably, from about 7 to about 35 nucleotides or nucleotide analogs, most preferably from about 15 to about 25 nucleotide or nucleotide analogs.

Preferably, the antisense oligonucleotides comprise from 3 to about 50 nucleotides or nucleotide analogs, more preferably from 20 to about 50 nucleotides or nucleotide analogs and further comprise all or part of the sequences set forth in Tables 1, 2, 3, and 4 (below). Preferably, the oligonucleotides complementary to the ribonucleotide reductase gene comprise SEQ ID NOS.: 14 to 157 as shown in Tables 1 and 2. Preferably, the antisense oligonucleotides complementary to the *secA* gene comprise the SEQ ID NOS.: 158 to 265 as shown in Tables 3 and 4.

Table 1

Antisense oligonucleotides that target the *Escherichia coli* K12 ribonucleotide reductase large subunit (R1)

SEQ ID No:	Name	Sequence 5'-3'	Tm (°C)	ΔG (kcal/mol)
14	ER1-16	CCGTCGCGCTTTGTCACCAG	61.1	-43.0
15	ER1-24	CTGTGCTACCGTCGCGCTTT	57.8	-42.0
16	ER1-33	TGATGCGCTCTGTGCTACCG	57.2	-40.2
17	ER1-44	TTTGTGCGAGATTGAT GCGCT	53.3	-38.7
18	ER1-58	AGAACGCGATGGATTTTGTC	51.7	-38.4
19	ER1-71	TGCCGCCCAATCCAGAACGC	64.6	-46.0
20	ER1-79	AGTCCTTCTGCCGCCCAATC	57.7	-42.2
21	ER1-128	AAACTGAATGTGGGAGCGCA	55.5	-39.8
22	ER1-169	ATAATGGTTTCGTGGATGTC	55.5	-35.4
23	ER1-180	CGGCAGCCTTGATAATGGTT	54.2	-40.6
24	ER1-218	ATACTGATAATCCGGCGCAT	51.4	-39.4
25	ER1-252	TACGCAGGTGGAAGATCGCC	57.3	-41.4

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SEQ ID No:	Name	Sequence 5'-3'	Tm (°C)	ΔG (kcal/mol)
26	ER1-294	GGTCGTACAGCGCAGGCGGC	64.4	-45.9
27	ER1-320	GCCCATCTCGACCATTTTCA	54.7	-39.7
28	ER1-330	TATCGTATTTGCCATCTCG	50.4	-38.1
29	ER1-423	CGGCAGCATAAGAGAAGGTC	51.6	-38.5
30	ER1-439	CCTTCCAGCTGCTTAACGGC	56.4	-41.9
31	ER1-450	CCAGATATTTGCCTTCCAGC	51.5	-38.8
32	ER1-479	ATAGATTTCGCCGGTCACGC	56.4	-41.8
33	ER1-495	GGAAGTGGGCGCTCTCATAG	53.9	-39.7
34	ER1-504	GAATATAAAGGAACTGGGCG	48.5	-38.0
35	ER1-518	GCACGCGGCAACTAGAAATAT	52.2	-39.4
36	ER1-529	TTCGAGAACAAGCACGCGGC	60.8	-43.3
37	ER1-543	TTTCACGCGGGTAGTTCGAG	55.2	-40.5
38	ER1-566	ACGCTTCACATATTGCAGGC	52.2	-38.7
39	ER1-584	GGAAACCGCGTCGTAAAAAC	53.9	-40.8
40	ER1-592	TTAAATGTGGAAACCGCGTC	52.7	-39.3
41	ER1-617	CATGATTGGCGTCGGCAGCG	64.0	-44.9
42	ER1-628	CGCACGCCGGACATGATTGG	63.8	-44.6
43	ER1-640	CGAGTCGGGGTACGCACGCC	64.2	-45.8
44	ER1-667	TCGATCAGTACGCAGGAGCT	52.4	-38.1
45	ER1-680	GCTGTCACCGCACTCGATCA	56.9	-39.1
46	ER1-689	GGAATCCAGGCTGTCACCGC	59.0	-41.9

SEQ ID No:	Name	Sequence 5'-3'	Tm (°C)	ΔG (kcal/mol)
47	ER1-704	GGAGGTGGCGTTGATGGAAT	56.0	-40.6
48	ER1-716	AACAATCGCGCTGGAGGTGG	59.5	-42.7
49	ER1-778	CTACCCAGCGCACGAATACG	55.7	-40.9
50	ER1-817	ATGCAGCCGGTATGGAACGC	59.4	-43.1
51	ER1-829	TTGTAGAACGGAATGCAGCC	52.8	-38.8
52	ER1-846	CCGCTGTCTGGAAATGTTTG	53.1	-38.6
53	ER1-855	AGGATTTACCGCTGTCTGG	54.0	-39.2
54	ER1-874	CGCACACCGCCCTGAGAGCA	63.9	-44.0
55	ER1-907	CACATCGGGTAGAACAGCGT	52.5	-38.1
56	ER1-925	CTTTCCACTTCCAGATGCCA	52.5	-38.1
57	ER1-964	TTGCCTTCCACACCACGGTT	57.5	-40.8
58	ER1-971	CACGCGGTTGCCTTCCACAC	60.8	-42.5
59	ER1-981	CCATATGACGCACGCGGTTG	59.4	-42.1
60	ER1-1034	TTCACCTTTCAGCAGACGGG	55.0	-39.6
61	ER1-1055	CGGGCTGAACAGGGTGATAT	53.8	-39.6
62	ER1-1059	CGGACGGGCTGAACAGGGTG	62.1	-43.7
63	ER1-1061	GTCGGACGGGCTGAACAGGG	61.2	-43.4
64	ER1-1106	AAACTCTTCCTGATCGGCGA	53.8	-39.7
65	ER1-1148	GCGGATGCTGTCGTCTTTCT	54.3	-39.4
66	ER1-1155	GCTGCTTGCGGATGCTGTCG	61.3	-43.0
67	ER1-1166	GGCTTTCACACGCTGCTTGC	58.2	-41.4

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SEQ ID No:	Name	Sequence 5'-3'	Tm (°C)	ΔG (kcal/mol)
68	ER1-1173	GCTCAACGGCTTTCACACGC	58.0	-41.3
69	ER1-1212	GACCGGTAGACGCACGTTCC	56.7	-40.8
70	ER1-1255	GGGCTATGGGTATTGCAGTG	52.1	-38.7
71	ER1-1259	AAACGGGCTATGGGTATTGC	53.3	-40.7
72	ER1-1265	CGGATCAAACGGGCTATGGG	58.7	-43.4
73	ER1-1311	GGGCTATCTCCAGGCACAGG	55.9	-40.7
74	ER1-1315	GGCAGGGCTATCTCCAGGCA	58.7	-42.5
75	ER1-1320	TGGTCGGCAGGGCTATCTCC	58.6	-42.4
76	ER1-1326	GCGGTTTGGTCGGCAGGGCT	64.9	-47.0
77	ER1-1330	TTCAGCGGTTTGGTCGGCAG	60.5	-43.1
78	ER1-1336	ACGTCGTTTCAGCGGTTTGGT	56.8	-40.9
79	ER1-1356	TTTACCGTTCTCGTCGTTG	53.5	-38.5
80	ER1-1364	CAGCGCGATTTCACCGTTCT	57.5	-41.7
81	ER1-1370	CGTACACAGCGCGATTTCAC	54.2	-38.9
82	ER1-1379	AGCAGACAGCGTACACAGCG	54.0	-38.2
83	ER1-1388	CAGGTTGAAAGCAGACAGCG	53.4	-38.4
84	ER1-1397	AATTGCGCCCAGGTTGAAAG	56.5	-41.9
85	ER1-1407	CCAGGTTATTAATTGCGCCC	53.8	-41.3
86	ER1-1428	TTGCCAGCTCTTCCAGTTCA	53.3	-38.2
87	ER1-1438	ACCGCCAGAATTGCCAGCTC	58.8	-42.5
88	ER1-1451	GTCAAGTGCACGAACCGCCA	59.1	-41.0

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SEQ ID No:	Name	Sequence 5'-3'	Tm (°C)	ΔG (kcal/mol)
89	ER1-1463	ATCCAGCAGCGCGTCAAGTG	58.5	-41.2
90	ER1-1468	TGATAATCCAGCAGCGCGTC	56.1	-40.4
91	ER1-1535	GATCACACCAATACCCAGCG	52.6	-38.1
92	ER1-1561	TCGTTCGCCAGGTAGTAAGC	52.2	-39.0
93	ER1-1570	CGTTTACCGTCGTTGCCAG	57.9	-42.2
94	ER1-1584	TGCCGTCGGAGTAGCGTTTA	55.8	-41.0
95	ER1-1605	TATGCGTCAGGTTGTTGGCG	56.8	-40.5
96	ER1-1614	CGAAGGTTTTATGCGTCAGG	52.5	-39.3
97	ER1-1688	GTAAACCACGGGCACGCGC	62.0	-45.0
98	ER1-1705	TTCGCGTAAGTGGTTTCGTT	52.6	-39.3
99	ER1-1731	TATAGGTATCGATCGGCAGG	49.5	-38.0
100	ER1-1777	CAGTCGTAATGCAGCGGCTC	55.8	-40.2
101	ER1-1789	CGCAGAGCTTCCCAGTCGTA	55.4	-40.0
102	ER1-1839	TCAGAGCAGAAAGCGTGGAG	53.0	-38.1
103	ER1-1849	TCGGACGGCATCAGAGCAGA	58.9	-40.9
104	ER1-1874	GCGGTTAGAGATCTGCGAAG	51.8	-38.7
105	ER1-1916	TTTGATGCTGACGTAACCGC	53.7	-39.0
106	ER1-1923	TCGACGCTTTGATGCTGACG	57.1	-40.2
107	ER1-1944	CCTGGCGCAAATACCGTCT	56.5	-42.0
108	ER1-1957	TAGTCCGGCACCACCTGGCG	62.5	-44.2
109	ER1-1968	GCAGGTGCTCGTAGTCCGGC	59.3	-42.4

SEQ ID No:	Name	Sequence 5'-3'	Tm (°C)	ΔG (kcal/mol)
110	ER1-1974	CGTCGTGCAGGTGCTCGTAG	56.7	-39.9
111	ER1-1983	GCTCATAGGCGTCGTGCAGG	58.0	-41.4
112	ER1-1992	CCCACAGCAGCTCATAGGCG	58.0	-41.5
113	ER1-2000	CGGCATTTCACAGCAGCT	59.7	-42.8
114	ER1-2010	CATCGTTACCCGGCATTTC	56.5	-41.9
115	ER1-2083	GGATCGTAGTTGGTGTGGC	51.8	-39.9
116	ER1-2112	TCGGCACTTTTCCTGACGGG	59.5	-42.8
117	ER1-2145	AGGCGGTGAGCAGGTCTTTC	55.7	-40.5
118	ER1-2154	CGAATTTGTAGGCGGTGAGC	54.8	-40.5
119	ER1-2166	GTGTTTTGACCCGAATTTG	51.9	-38.6
120	ER1-2211	CGTCTTGTGCGTCTTCAGCG	56.8	-40.0
121	ER1-2262	TCTTACATGCGCCGCTTTCG	58.6	-42.8

Table 2
Antisense oligonucleotides that target the *Escherichia coli* K12 ribonucleotide reductase small subunit (R2)

SEQ ID No:	Name	Sequence 5'-3'	Tm (°C)	ΔG (kcal/mol)
122	ER2-50	CGGCTGACCAAAGAACATCG	55.5	-40.0
123	ER2-60	CCACGTTGACCGGCTGACCA	61.2	-42.2
124	ER2-67	TAGCGAGCCACGTTGACCGG	60.6	-43.2
125	ER2-134	CGGACGCCAGAAGAAAGAGA	54.4	-39.8
126	ER2-144	CAACTTCTTCCGGACGCCAG	57.0	-41.3

SEQ ID No:	Name	Sequence 5'-3'	Tm (°C)	ΔG (kcal/mol)
127	ER2-168	AATCTATACGGTCGCGGGAG	53.4	-40.5
128	ER2-198	TGTGTTTTTCGTGCTCCGGC	58.3	-41.6
129	ER2-273	GCAATAGCGCCACGTTCGGG	62.1	-45.2
130	ER2-284	AGAAATAAGCGGCAATAGCG	51.8	-40.3
131	ER2-290	CGGAATAGAAATAAGCGGCA	52.4	-40.3
132	ER2-307	ACCCAGGTTTCCAGTTCGGG	57.4	-42.0
133	ER2-350	ATAGGAACGGGAATGAATCG	50.7	-38.8
134	ER2-441	TCCCTTCCGCACGTTTCTGG	59.5	-42.8
135	ER2-498	CGCCCAGCAGATGCCAGTAG	58.0	-41.5
136	ER2-505	GTACCTTCGCCCAGCAGATG	54.6	-39.7
137	ER2-544	CGCAGGCTAACGGTCACAGT	55.2	-39.7
138	ER2-557	TTTCTTCAGCTCGCGCAGGC	60.2	-43.4
139	ER2-640	GCAAATGCGAAGGAACAAGC	54.9	-40.4
140	ER2-655	ATCAATTCGCGTTCTGCAA	53.4	-39.3
141	ER2-680	GCGAATAATTTTGGCGTTGC	54.9	-41.6
142	ER2-692	GCGGGCAATCAGGCGAATAA	59.5	-44.0
143	ER2-704	CAGGGCTTCGTGCGGGCAA	66.8	-47.8
144	ER2-714	CGGTCAGGTGCAGGGCTTCG	62.3	-44.0
145	ER2-724	TGCTGGGTGCCGGTCAGGTG	63.6	-43.5
146	ER2-728	CATATGCTGGGTGCCGGTCA	58.8	-41.4
147	ER2-778	GCAATTTCCGCCATCTCAGG	56.8	-41.5

SEQ ID No:	Name	Sequence 5'-3'	Tm (°C)	ΔG (kcal/mol)
148	ER2-796	TCCTGCTTACACTCTTCGGC	52.1	-38.3
149	ER2-848	ATCCGCCCAGTCTTTCTCCT	54.2	-40.4
150	ER2-857	GAACAGATAATCCGCCCAGT	50.7	-38.1
151	ER2-976	GGGTTGGAGCGCGTCTGGAA	61.8	-44.0
152	ER2-983	CGGGATCGGGTTGGAGCGCG	68.1	-49.1
153	ER2-985	CACGGGATCGGGTTGGAGCG	64.0	-45.6
154	ER2-1045	CTGACTTCCACTTCCTGCGG	54.6	-39.9
155	ER2-1063	TGCCCCGACCAGATAAGAACT	51.3	-38.2
156	ER2-1076	TTCCGAGTCAATCTGCCCCGA	57.8	-41.2
157	ER2-1092	AATCGTCGGTGTCCACTTCC	53.6	-38.8

Table 3
Antisense Sequences that Target *Escherichia coli* SecA

SEQ ID No:	Name	Sequence 5 - 3'	Tm (°C)	ΔG kDa/mol
158	ES56	GACCACTTTGCGCATCCGGC	62.1	-44.2
159	ES62	GATGTTGACCACTTTGCGCA	54.3	-38.3
160	ES85	ATCTCCGGTTCATGGCATT	55.5	-40.8
161	ES92	TTTTTCCATCTCCGGTCCA	54.3	-40.1
162	ES116	CCCTTTCAGTTCTTCGTCGG	53.8	-39.8
163	ES124	GCGGTTTTCCCTTTCAGTTC	52.9	-39.9
164	ES129	ACTCTGCGGTTTTCCCTTTC	52.5	-39.6
165	ES153	CGCCTTTTTCCAGACGTGCA	58.4	-41.9
166	ES158	CACTTCGCCTTTTTCCAGAC	51.5	-38.4
167	ES165	TTCCAGCACTTCGCCTTTT	54.1	-40.5

SEQ ID No:	Name	Sequence 5 - 3'	Tm (°C)	ΔG kDa/mol
168	ES170	CAGATTTTCCAGCACTTCGC	52.5	-38.6
169	ES206	ACTTGCCTCACGTACCACGG	54.9	-39.5
170	ES215	GACGCGCTTACTTGCCTCAC	55.0	-40.1
171	ES230	GTGACGCATACCAAAGACGC	53.1	-38.5
5 172	ES264	TAAGAACCATACCGCCGAGT	51.5	-39.1
173	ES286	ATTTTCGGCGATGCAGCGTTC	59.7	-43.4
174	ES303	TTCCTTCACCGGTACGCATT	54.5	-40.3
175	ES307	GTTTTTCCTTCACCGGTACG	51.4	-38.9
176	ES320	CGTTGCGGTCAGGGTTTTTC	56.8	-41.6
10 177	ES336	TCAGGTAAGCAGGCAGCGTT	55.0	-40.2
178	ES351	TACCGGTTAGTGCGTTCAGG	52.8	-39.2
179	ES392	TTGCGCCAGGTAGTCGTTGA	56.5	-40.4
180	ES398	GTCACGTTGCGCCAGGTAGT	55.0	-39.5
181	ES418	AGCGGACGGTTGTTTTTCGGC	60.8	-44.5
15 182	ES429	GGAATTCAAACAGCGGACGG	56.7	-41.5
183	ES436	AGGCCAAGGAATTCAAACAG	51.0	-38.4
184	ES448	ATACCGACAGTCAGGCCAAG	51.6	-38.0
185	ES485	TTCGCGCTTTGCCGGTGCTG	65.8	-46.9
186	ES531	AGCCGTATTCGTTGTTTCGTA	50.1	-37.9
20 187	ES544	CGCAGGTAGTCAAAGCCGTA	53.1	-39.5
188	ES553	ATGTTGTGCGCGCAGGTAGTC	52.6	-38.1
189	ES556	GCCATGTTGTGCGCGCAGGTA	59.2	-41.7
190	ES617	GTCCACTTCGTCCACCAGCG	57.7	-40.4
191	ES646	GGTGTACGCGCTTCATCGAT	55.0	-40.0
25 192	ES647	CGGTGTACGCGCTTCATCGA	59.3	-42.1
193	ES695	GCGTTTATACATTTCCGAGC	49.5	-38.4
194	ES724	CGGATCAGGTGCGGAATAAT	53.9	-40.4

SEQ ID No:	Name	Sequence 5' - 3'	Tm (°C)	ΔG kDa/mol
195	ES799	TTCACCTGGCGAGATTTTTC	51.8	-38.6
196	ES824	CAGCACCAGACCACGTTTCGG	58.6	-40.7
197	ES851	GCCCTCTTTCACCAGCAGTT	53.3	-39.1
198	ES866	CCCTTCATCCATGATGCCCT	55.9	-40.6
199	ES889	TTGGCCGGAGAGTACAGAGA	52.2	-38.1
200	ES898	AGCATGATGTTGGCCGGAGA	57.6	-40.9
201	ES922	AGCGCCGCCGTTACGTGGTG	64.6	-46.5
202	ES950	GTCACGGGTAAACAGCGCAT	54.9	-40.0
203	ES1068	CACCTTCTTTCGCTTCCACA	52.8	-38.4
204	ES1097	CAGCGTTTGGTTTTCGTTCT	52.1	-38.9
205	ES1109	GGTGATCGAAGCCAGCGTTT	56.5	-41.2
206	ES1128	GACGGAAGTAGTTCTGGAAG	45.5	-35.0
207	ES1147	CCCGCCAGTTTTTCATACAG	52.3	-39.2
208	ES1152	TCATCCCCGCCAGTTTTTCA	57.5	-41.6
209	ES1218	GAACAACGACGGTATCCAGC	52.0	-38.2
210	ES1328	GCCTTTCGCAGTACGTTCTT	51.4	-38.9
211	ES1350	TAGTACCCACCAGCACCGGC	57.1	-41.4
212	ES1398	CGGCTTTGGTCAGTTCGTTT	54.3	-40.1
213	ES1410	TGTGCTTAATACCGGCTTTG	50.8	-38.6
214	ES1439	GTTGGCGTGGAATTTGGCGT	59.3	-43.0
215	ES1462	GCCTGAGCAACAATCGCCGC	62.4	-44.5
216	ES1515	CTGTACCACGACCCGCCATA	55.6	-40.3
217	ES1518	TATCTGTACCACGACCCGCC	54.7	-40.0
218	ES1545	CTGCCTGCCAGCTACCACCG	60.2	-42.9
219	ES1563	TTTCCAGCGCGGCAACTTCT	59.4	-43.4
220	ES1581	TTTGCTCTGCGGTCGGATTT	57.0	-41.8
221	ES1589	TTTTTCAATTTGCTCTGCGG	53.2	-39.8

SEQ ID No:	Name	Sequence 5' - 3'	Tm (°C)	ΔG kDa/mol
222	ES1624	ACCGCATCGTGACGTACCTG	55.7	-39.6
223	ES1629	CCAGTACCGCATCGTGACGT	55.7	-39.6
224	ES1633	GCTTCCAGTACCGCATCGTG	55.5	-40.0
225	ES1655	ACCGATGATATGCAGGCCAC	54.6	-39.6
226	ES1712	ACGACCAGAACGACCGCGCA	63.3	-44.1
227	ES1718	CCCCTGACGACCAGAACGAC	56.6	-40.1
228	ES1722	CATCCCCCTGACGACCAGAA	56.9	-40.4
229	ES1739	GAAACGGGAAGAACCAGCAT	53.1	-39.5
230	ES1748	CGACAGGTAGAAACGGGAAG	51.4	-38.6
231	ES1781	GGAAGCAAAAATACGCATCA	50.6	-38.2
232	ES1785	GGTCGGAAGCAAAAATACGC	53.9	-40.9
233	ES1794	CGGATACTCGGTCGGAAGCA	57.3	-41.7
234	ES1814	ACCCAGTTTACGCATCATGC	52.5	-38.5
235	ES1845	ACGGGTGTTCAATGGCTTCG	57.1	-41.2
236	ES1861	ATCGCTTTAGTCACCCACGG	54.1	-40.0
237	ES1888	CTTTCAACTTTACGCTGGGC	51.9	-39.3
238	ES1892	ACGGCTTTCAACTTTACGCT	51.1	-39.2
239	ES2007	TGGTTTCGCTCACATCGCTG	57.0	-40.0
240	ES2054	GTAGGCATCAATGGTCGCTT	51.7	-38.5
241	ES2084	CCACATTTCTTCCAGCGACT	51.7	-38.0
242	ES2087	ATCCCACATTTCTTCCAGCG	53.9	-39.7
243	ES2191	TCACGCAGCGTCTCTTCATG	54.7	-38.2
244	ES2275	CCTTTCTCGAAGTGACGCAT	51.9	-38.2
245	ES2306	CCACAGGGAGTCAAGCGTTT	54.1	-39.3
246	ES2325	TCGCTGCCAGGTGCTCTTTC	57.7	-41.1
247	ES2330	GTCCATCGCTGCCAGGTGCT	59.7	-41.9
248	ES2339	ACGCAGATAGTCCATCGCTG	52.7	-38.4

SEQ ID No:	Name	Sequence 5 - 3'	Tm (°C)	ΔG kDa/mol
249	ES2381	CTTCGGATCTTTCTGTGCGT	51.9	-38.2
250	ES2395	CGTTTGTATTCCTGCTTCGG	52.5	-39.4
251	ES2422	ATCGCTGCAAACATGGAGAA	53.1	-38.5
252	ES2520	CCATACGACGCTGTTGTTCC	52.9	-38.5
253	ES2525	GGCTTCCATACGACGCTGTT	54.2	-40.0
254	ES2537	CGCTAAACGCTCGGCTTCCA	59.9	-44.1
255	ES2555	GCTAAGCTGCTGCATTTGCG	56.2	-41.3
256	ES2619	CTACTTTGCGCTCTCCGGTT	53.8	-40.4
257	ES2626	TTACGTCCTACTTTGCGCTC	50.0	-38.0
258	ES2646	AACCGCACGGGCAAGGATCG	63.6	-45.9
259	ES2651	ACCAGAACCGCACGGGCAAG	61.7	-44.0
260	ES2656	TTTTTACCAGAACCGCACGG	55.1	-41.0

Table 4
Antisense Sequences that Target *E. coli* *SecA* based on Conserved Sequences

SEQ ID No:	Name	Sequence 5 - 3'	Tm (°C)	ΔG kDa/mol
261	ES386	CAGGTAGTCGTTGACGGTAA	47.7	-35.7
262	ES388	CAGGTAGTCGTTGACGGT	45.0	-32.9
263	ES1126	CGGAAGTAGTTCTGGAAGGT	47.6	-36.5
264	ES1702	CGACCGCGCAACTGGTTATC	57.8	-41.9
265	ES2644	CCGCACGGGCAAGGATCGTT	63.6	-45.9

In Tables 1, 2, 3, and 4, the "Tm" is the melting temperature of an oligonucleotide duplex calculated according to the nearest-neighbor thermodynamic values. At this temperature 50% of nucleic acid molecules are in duplex and 50% are denatured. The "ΔG" is the free energy of the oligonucleotide, which is a measurement of an oligonucleotide duplex stability.

The following sequences have been determined to be conserved among species:

ES386 [SEQ ID NO:261] is conserved among *Escherichia coli* and
Mycobacterium tuberculosis;

ES388 [SEQ ID NO:262] is conserved among *Escherichia coli*; *Mycobacterium*
5 *tuberculosis*; and *Mycobacterium bovis*;

ES553 [SEQ ID NO:188] is conserved among *Escherichia coli*, *Mycobacterium*
tuberculosis, *Mycobacterium bovis*, *Streptomyces coelicolor*; and *Streptomyces lividans*;

ES556 [SEQ ID NO:189] is conserved among *Escherichia coli*, *Mycobacterium*
tuberculosis, *Mycobacterium bovis*, *Streptomyces coelicolor*; and *Streptomyces lividans*;
10 and *Synechococcus sp.*; and

ES646 [SEQ ID NO:191] is conserved among *Escherichia coli* and
Staphylococcus carnosus;

ES1126 [SEQ ID NO:263] is conserved among *Escherichia coli* and
Rhodobacter capsulatus SecA genes.

15 ES2644 [SEQ ID NO:265] is conserved among *Escherichia coli* SecA gene,
MutA (A:T to C:G transversion), and tyrosine-specific transport protein (tyrP) gene.

The term "alkyl" refers to monovalent alkyl groups preferably having from 1 to
20 carbon atoms and more preferably 1 to 6 carbon atoms. This term is exemplified
by groups such as methyl, ethyl, *n*-propyl, *iso*-propyl, *n*-butyl, *iso*-butyl, *n*-hexyl, and
20 the like.

The term "aryl" refers to an unsaturated aromatic carbocyclic group of from 6
to 14 carbon atoms having a single ring (e.g., phenyl) or multiple condensed (fused)
rings (e.g., naphthyl or anthryl). Preferred aryls include phenyl, naphthyl and the like.

The term "cycloalkyl" refers to cyclic alkyl groups of from 3 to 20 carbon
25 atoms having a single cyclic ring or multiple condensed rings. Such cycloalkyl groups
include, by way of example, single ring structures such as cyclopropyl, cyclobutyl,
cyclopentyl, cyclooctyl, and the like, or multiple ring structures such as adamantanyl,
and the like.

The term "halo" or "halogen" refers to fluoro, chloro, bromo and iodo and
30 preferably is either fluoro or chloro.

The term "thiol" refers to the group -SH.

As to any of the above groups which contain one or more substituents, it is understood, of course, that such groups do not contain any substitution or substitution patterns which are sterically impractical and/or synthetically non-feasible. In addition, the compounds of this invention include all stereochemical isomers arising from the substitution of these compounds.

The term "pharmaceutically acceptable salt" refers to salts which retain the biological effectiveness and properties of the antisense oligonucleotides of this invention and which are not biologically or otherwise undesirable. In many cases, the antisense oligonucleotides of this invention are capable of forming acid and/or base salts by virtue of the presence of amino and/or carboxyl groups or groups similar thereto.

Pharmaceutically acceptable base addition salts can be prepared from inorganic and organic bases. Salts derived from inorganic bases, include by way of example only, sodium, potassium, lithium, ammonium, calcium and magnesium salts. Salts derived from organic bases include, but are not limited to, salts of primary, secondary and tertiary amines, such as alkyl amines, dialkyl amines, trialkyl amines, substituted alkyl amines, di(substituted alkyl) amines, tri(substituted alkyl) amines, alkenyl amines, dialkenyl amines, trialkenyl amines, substituted alkenyl amines, di(substituted alkenyl) amines, tri(substituted alkenyl) amines, cycloalkyl amines, di(cycloalkyl) amines, tri(cycloalkyl) amines, substituted cycloalkyl amines, disubstituted cycloalkyl amine, trisubstituted cycloalkyl amines, cycloalkenyl amines, di(cycloalkenyl) amines, tri(cycloalkenyl) amines, substituted cycloalkenyl amines, disubstituted cycloalkenyl amine, trisubstituted cycloalkenyl amines, aryl amines, diaryl amines, triaryl amines, heteroaryl amines, diheteroaryl amines, triheteroaryl amines, heterocyclic amines, diheterocyclic amines, triheterocyclic amines, mixed di- and tri-amines where at least two of the substituents on the amine are different and are selected from the group consisting of alkyl, substituted alkyl, alkenyl, substituted alkenyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, heteroaryl,

heterocyclic, and the like. Also included are amines where the two or three substituents, together with the amino nitrogen, form a heterocyclic or heteroaryl group.

Examples of suitable amines include, by way of example only, isopropylamine, trimethyl amine, diethyl amine, tri(*iso*-propyl) amine, tri(*n*-propyl) amine, 5 ethanolamine, 2-dimethylaminoethanol, tromethamine, lysine, arginine, histidine, caffeine, procaine, hydrabamine, choline, betaine, ethylenediamine, glucosamine, N-alkylglucamines, theobromine, purines, piperazine, piperidine, morpholine, N-ethylpiperidine, and the like. It should also be understood that other carboxylic acid derivatives would be useful in the practice of this invention, for example, carboxylic 10 acid amides, including carboxamides, lower alkyl carboxamides, dialkyl carboxamides, and the like.

Pharmaceutically acceptable acid addition salts may be prepared from inorganic and organic acids. Salts derived from inorganic acids include hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like. Salts 15 derived from organic acids include acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, malic acid, malonic acid, succinic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, *p*-toluene-sulfonic acid, salicylic acid, and the like.

The term "ribonucleotide reductase gene" or the "ribonucleoside diphosphate 20 reductase gene" refers to any gene which encodes a protein that either reduces the four main ribonucleotides to the corresponding deoxyribonucleotides involved in DNA synthesis or encodes a subunit of a multimeric enzyme which reduces the four main ribonucleotides to the corresponding deoxyribonucleotides. Without being limiting, examples of ribonucleotide reductase genes from bacteria include the *E. coli* *nrdA*, 25 *nrdB* and *nrdD* genes; the *S. typhimurium* *nrdE* and *nrdF* genes; and the *Lactococcus lactis* *nrdEF* gene. Examples of the ribonucleotide reductase genes from viruses include the herpes simplex type 1 and 2 ribonucleotide reductases and the bovine and equine herpes simplex ribonucleotide reductases.

The term "secA" refers to an oligonucleotide sequence which encodes a protein 30 having similar properties as those expressed by the *E. coli* *secA* gene. Without being

limiting, examples of secA genes from bacteria include the *Mycobacterium bovis* secA gene; the *Mycobacterium tuberculosis* secA gene, the *Staphylococcus aureus* secA gene and the *Staphylococcus carnosus* secA gene.

5 The term "microorganism" means a bacteria, fungi or virus having either a ribonucleotide reductase or secA gene. Specifically excluded from this definition is the malarial parasite, plasmodium.

The term "bacteria" refers to any bacteria encoding either a ribonucleotide reductase gene or a secA gene, including *Escherichia coli*, *Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Mycobacterium smegmatis*, *Salmonella typhimurium*,
10 *Thermoplasma acidophilum*, *Pyrococcus furiosus*, *Bacillus subtilis*, *Bacillus firmus*, *Lactococcus lactis*, *Staphylococcus aureus*, *Staphylococcus carnosus*, *Listeria monocytogenes*, *Borrelia burgdorferi*, *P. sativum*, *S. griseus*, and *Synechococcus* sp.

The term "virus" refers to any virus having a ribonucleotide reductase gene. Preferably the virus will be a DNA virus. Examples of suitable viruses include various
15 herpes viruses (such as herpes simplex types 1 and 2, varicella-herpes zoster, cytomegalovirus and Epstein-Barr virus) and the various hepatitis viruses.

The term "complementary to" means that the antisense oligonucleotide sequence is capable of binding to the target sequence, ie the ribonucleotide reductase gene or the secA gene. Preferably the antisense oligonucleotide sequence has at least about 75%
20 identity with the target sequence, preferably at least about 90% identity and most preferably at least about 95% identity with the target sequence allowing for gaps or mismatches of several bases. Identity can be determined, for example, by using the BLASTN program of the University of Wisconsin Computer Group (GCG) software.

The term "inhibiting growth" means a reduction in the growth of the bacteria or
25 viruses of at least 25%, more preferably of at least 50% and most preferably of at least 75%. The reduction in growth can be determined for bacteria by measuring the optical density of a liquid bacteria culture with a spectrophotometer or by counting the number of colony forming units/ml (CFU/ml) upon plating on culture plates. The reduction in growth can be determined for viruses by measuring the number of plaque
30 forming units/ml upon plating on susceptible cells.

Preparation of the Antisense Oligonucleotides

The antisense oligonucleotides of the present invention may be prepared by conventional and well-known techniques. For example, the oligonucleotides may be prepared using solid-phase synthesis and in particular using commercially available equipment such as the equipment available from Applied Biosystems Canada Inc., Mississauga, Canada. The oligonucleotides may also be prepared by enzymatic digestion of the naturally occurring ribonucleotide reductase or secA gene by methods known in the art.

Isolation and Purification of the Antisense Oligonucleotides

Isolation and purification of the antisense oligonucleotides described herein can be effected, if desired, by any suitable separation or purification such as, for example, filtration, extraction, crystallization, column chromatography, thin-layer chromatography, thick-layer chromatography, preparative low or high-pressure liquid chromatography or a combination of these procedures. However, other equivalent separation or isolation procedures could, of course, also be used.

The invention contemplates a method of evaluating if an antisense oligonucleotide inhibits the growth of a microbe having a ribonucleotide reductase or secA gene. The method comprises selecting the microbe/microorganism having a ribonucleotide reductase or secA gene, administering the antisense oligonucleotide; and comparing the growth of the treated microbe with the growth of an untreated microorganism.

In order for the antisense oligonucleotide to effectively interrupt the expression of the ribonucleotide reductase or secA gene, the antisense oligonucleotide enters the microorganism's cell, in the case of fungal or bacterial cells or enter the mammalian cell having the virus target.

Although oligonucleotides are taken up by bacterial cells, some modification of the oligonucleotides may help facilitate or regulate said uptake. thus, a carrier molecule, for example an amino acid, can be linked to the oligonucleotide. for example, bacteria have multiple transport systems for the recognition and uptake of

molecules of leucine. The addition of this amino acid to the oligonucleotide may facilitate the uptake of the oligonucleotide in the bacteria and not substantially interfere with the activity of the antisense oligonucleotide in the bacterial cell.

Other methods are contemplated for facilitating the uptake of the antisense oligonucleotide into bacteria. For example, the addition of other amino acids or peptides or primary amines to the 3' or 5' termini of the antisense oligonucleotide may enable utilization of specific transport systems. Addition of lactose to the oligonucleotide by a covalent linkage may also be used to enable transport of the antisense oligonucleotide by lactose permease. Other sugar transport systems are also known to be functional in bacteria and can be utilized in this invention.

With regard to inhibiting the expression of ribonucleotide reductase in DNA viruses, the antisense oligonucleotide is preferably introduced into the cell infected with the DNA virus. The antisense oligonucleotides may be delivered using vectors or liposomes.

An expression vector comprising the antisense oligonucleotide sequence may be constructed having regard to the sequence of the oligonucleotide and using procedures known in the art. The vectors may be selected from plasmids or benign viral vectors depending on the eukaryotic cell and the DNA virus. Phagemids are a specific example of beneficial vectors because they can be used either as plasmids or a bacteriophage vectors. Examples of other vectors include viruses such as bacteriophages, baculoviruses and retroviruses, DNA viruses, liposomes and other recombination vectors.

Vectors can be constructed by those skilled in the art to contain all the expression elements required to achieve the desired transcription of the antisense oligonucleotide sequences. Therefore, the invention provides vectors comprising a transcription control sequence operatively linked to a sequence which encodes an antisense oligonucleotide. Suitable transcription and translation elements may be derived from a variety of sources, including bacterial, fungal, viral, mammalian or insect genes. Selection of appropriate elements is dependent on the host cell chosen.

Reporter genes may be included in the vector. Suitable reporter genes include β -galactosidase (e.g. lacZ), chloramphenicol, acetyl-transferase, firefly luciferase, or an immunoglobulin or portion thereof. Transcription of the antisense oligonucleotide may be monitored by monitoring for the expression of the reporter gene.

5 The vectors can be introduced into cells or tissues by any one of a variety of known methods within the art. Such methods can be found generally described in Sambrook et al.¹⁸; Ausubel et al.¹⁹; Chang et al.²⁰; Vega et al.²¹; and Vectors: A Survey of Molecular Cloning Vectors and Their Uses²² and include, for example, stable or transient transfection, lipofection, electroporation and infection with
10 recombinant viral vectors.

Introduction of nucleic acids by infection offers several advantages. Higher efficiency and specificity for tissue type can be obtained. Viruses typically infect and propagate in specific cell types. Thus, the virus' specificity may be used to target the vector to specific cell types *in vivo* or within a tissue or mixed culture of cells. Viral
15 vectors can also be modified with specific receptors or ligands to alter target specificity through receptor mediated events.

Pharmaceutical Formulations

When employed as pharmaceuticals, the antisense oligonucleotides are usually
20 administered in the form of pharmaceutical compositions. These compounds can be administered by a variety of routes including oral, rectal, transdermal, subcutaneous, intravenous, intramuscular, and intranasal. These compounds are effective as both injectable and oral compositions. Such compositions are prepared in a manner well known in the pharmaceutical art and comprise at least one active compound.

25 This invention also includes pharmaceutical compositions which contain, as the active ingredient, one or more of the antisense oligonucleotides associated with pharmaceutically acceptable carriers. In making the compositions of this invention, the active ingredient is usually mixed with an excipient, diluted by an excipient or enclosed within such a carrier which can be in the form of a capsule, sachet, paper or other
30 container. When the excipient serves as a diluent, it can be a solid, semi-solid, or

liquid material, which acts as a vehicle, carrier or medium for the active ingredient. Thus, the compositions can be in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols (as a solid or in a liquid medium), ointments containing, for example, up to 10% by weight of the active compound, soft and hard gelatin capsules, suppositories, sterile injectable solutions, and sterile packaged powders.

In preparing a formulation, it may be necessary to mill the active compound to provide the appropriate particle size prior to combining with the other ingredients. If the active compound is substantially insoluble, it ordinarily is milled to a particle size of less than 200 mesh. If the active compound is substantially water soluble, the particle size is normally adjusted by milling to provide a substantially uniform distribution in the formulation, e.g. about 40 mesh.

Some examples of suitable excipients include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, sterile water, syrup, and methyl cellulose. The formulations can additionally include: lubricating agents such as talc, magnesium stearate, and mineral oil; wetting agents; emulsifying and suspending agents; preserving agents such as methyl- and propylhydroxy-benzoates; sweetening agents; and flavoring agents. The compositions of the invention can be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the patient by employing procedures known in the art.

The compositions are preferably formulated in a unit dosage form, each dosage containing from about 5 to about 100 mg, more usually about 10 to about 30 mg, of the active ingredient. The term "unit dosage forms" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical excipient. Preferably, the antisense oligonucleotide is employed at no more than about 20 weight percent of

the pharmaceutical composition, more preferably no more than about 15 weight percent, with the balance being pharmaceutically inert carrier(s).

The antisense oligonucleotide is effective over a wide dosage range and is generally administered in a pharmaceutically effective amount. It, will be understood, however, that the amount of the antisense oligonucleotide actually administered will be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual compound administered, the age, weight, and response of the individual patient, the severity of the patient's symptoms, and the like.

For preparing solid compositions such as tablets, the principal active ingredient/antisense oligonucleotide is mixed with a pharmaceutical excipient to form a solid preformulation composition containing a homogeneous mixture of a compound of the present invention. When referring to these preformulation compositions as homogeneous, it is meant that the active ingredient is dispersed evenly throughout the composition so that the composition may be readily subdivided into equally effective unit dosage forms such as tablets, pills and capsules. This solid preformulation is then subdivided into unit dosage forms of the type described above containing from, for example, 0.1 to about 500 mg of the active ingredient of the present invention.

The tablets or pills of the present invention may be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, the tablet or pill can comprise an inner dosage and an outer dosage component, the latter being in the form of an envelope over the former. The two components can be separated by an enteric layer which serves to resist disintegration in the stomach and permit the inner component to pass intact into the duodenum or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, such materials including a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol, and cellulose acetate.

The liquid forms in which the novel compositions of the present invention may be incorporated for administration orally or by injection include aqueous solutions, suitably flavored syrups, aqueous or oil suspensions, and flavored emulsions with

edible oils such as corn oil, cottonseed oil, sesame oil, coconut oil, or peanut oil, as well as elixirs and similar pharmaceutical vehicles.

Compositions for inhalation or insufflation include solutions and suspensions in pharmaceutically acceptable, aqueous or organic solvents, or mixtures thereof, and
5 powders. The liquid or solid compositions may contain suitable pharmaceutically acceptable excipients as described *supra*. Preferably the compositions are administered by the oral or nasal respiratory route for local or systemic effect. Compositions in preferably pharmaceutically acceptable solvents may be nebulized by use of inert gases. Nebulized solutions may be inhaled directly from the nebulizing device or the
10 nebulizing device may be attached to a face mask tent, or intermittent positive pressure breathing machine. Solution, suspension, or powder compositions may be administered, preferably orally or nasally, from devices which deliver the formulation in an appropriate manner.

The following formulation examples illustrate representative pharmaceutical
15 compositions of the present invention.

Formulation Example 1

Hard gelatin capsules containing the following ingredients are prepared:

20	<u>Ingredient</u>	<u>Quantity</u> <u>(mg/capsule)</u>
	Active Ingredient	30.0
	Starch	305.0
25	Magnesium stearate	5.0

The above ingredients are mixed and filled into hard gelatin capsules in 340 mg quantities.

Formulation Example 2

A tablet formula is prepared using the ingredients below:

5	<u>Ingredient</u>	<u>Quantity</u> <u>(mg/tablet)</u>
	Active Ingredient	25.0
	Cellulose, microcrystalline	200.0
	Colloidal silicon dioxide	10.0
	Stearic acid	5.0
10	240 mg.	

The components are blended and compressed to form tablets, each weighing

Formulation Example 3

A dry powder inhaler formulation is prepared containing the following components:

15	<u>Ingredient</u>	<u>Weight %</u>
	Active Ingredient	5
	Lactose	95

The active ingredient is mixed with the lactose and the mixture is added to a dry powder inhaling appliance.

Formulation Example 4

Tablets, each containing 30 mg of active ingredient, are prepared as follows:

25	<u>Ingredient</u>	<u>Quantity</u> <u>(mg/tablet)</u>
	Active Ingredient	30.0 mg
	Starch	45.0 mg
	Microcrystalline cellulose	35.0 mg
30	Polyvinylpyrrolidone (as 10% solution in sterile water)	4.0 mg
	Sodium carboxymethyl starch	4.5 mg
	Magnesium stearate	0.5 mg
	Talc	<u>1.0 mg</u>
35	Total	120 mg

The active ingredient, starch and cellulose are passed through a No. 20 mesh U.S. sieve and mixed thoroughly. The solution of polyvinylpyrrolidone is mixed with the resultant powders, which are then passed through a 16 mesh U.S. sieve. The granules so produced are dried at 50° to 60°C and passed through a 16 mesh U.S. sieve. The sodium carboxymethyl starch, magnesium stearate, and talc, previously passed through a No. 30 mesh U.S. sieve, are then added to the granules which, after mixing, are compressed on a tablet machine to yield tablets each weighing 120 mg.

Formulation Example 5

Capsules, each containing 40 mg of medicament are made as follows:

<u>Ingredient</u>	<u>Quantity (mg/capsule)</u>
Active Ingredient	40.0 mg
Starch	109.0 mg
Magnesium stearate	<u>1.0 mg</u>
Total	150.0 mg

The active ingredient, starch, and magnesium stearate are blended, passed through a No. 20 mesh U.S. sieve, and filled into hard gelatin capsules in 150 mg quantities.

Formulation Example 6

Suppositories, each containing 25 mg of active ingredient are made as follows:

<u>Ingredient</u>	<u>Amount</u>
Active Ingredient	25 mg
Saturated fatty acid glycerides to	2,000 mg

The active ingredient is passed through a No. 60 mesh U.S. sieve and suspended in the saturated fatty acid glycerides previously melted using the minimum heat necessary. The mixture is then poured into a suppository mold of nominal 2.0 g capacity and allowed to cool.

Formulation Example 7

Suspensions, each containing 50 mg of medicament per 5.0 mL dose are made as follows:

5	<u>Ingredient</u>	<u>Amount</u>
	Active Ingredient	50.0 mg
	Xanthan gum	4.0 mg
	Sodium carboxymethyl cellulose (11%)	
	Microcrystalline cellulose (89%)	50.0 mg
10	Sucrose	1.75 g
	Sodium benzoate	10.0 mg
	Flavor and Color	q.v.
	Purified water to	5.0 mL

15 The active ingredient, sucrose and xanthan gum are blended, passed through a No. 10 mesh U.S. sieve, and then mixed with a previously made solution of the microcrystalline cellulose and sodium carboxymethyl cellulose in water. The sodium benzoate, flavor, and color are diluted with some of the water and added with stirring. Sufficient water is then added to produce the required volume.

20

Formulation Example 8

	<u>Ingredient</u>	<u>Quantity (mg/capsule)</u>
25	Active Ingredient	15.0 mg
	Starch	407.0 mg
	Magnesium stearate	<u>3.0 mg</u>
30	Total	425.0 mg

The active ingredient, starch, and magnesium stearate are blended, passed through a No. 20 mesh U.S. sieve, and filled into hard gelatin capsules in 425.0 mg quantities.

35

Formulation Example 9

A formulation may be prepared as follows:

	<u>Ingredient</u>	<u>Quantity</u>
5	Active Ingredient	5.0 mg
	Corn Oil	1.0 mL

Formulation Example 10

A topical formulation may be prepared as follows:

10	<u>Ingredient</u>	<u>Quantity</u>
	Active Ingredient	1-10 g
	Emulsifying Wax	30 g
15	Liquid Paraffin	20 g
	White Soft Paraffin	to 100 g

The white soft paraffin is heated until molten. The liquid paraffin and emulsifying wax are incorporated and stirred until dissolved. The active ingredient is added and stirring is continued until dispersed. The mixture is then cooled until solid.

Another preferred formulation employed in the methods of the present invention employs transdermal delivery devices ("patches"). Such transdermal patches may be used to provide continuous or discontinuous infusion of the antisense oligonucleotides of the present invention in controlled amounts. The construction and use of transdermal patches for the delivery of pharmaceutical agents is well known in the art. See, for example, U.S. Patent 5,023,252²³, herein incorporated by reference. Such patches may be constructed for continuous, pulsatile, or on demand delivery of pharmaceutical agents.

Another preferred method of delivery involves "shotgun" delivery of the naked antisense oligonucleotides across the dermal layer. The delivery of "naked" antisense oligonucleotides is well known in the art. See, for example, Felgner et al., U.S. Patent No. 5,580,859²⁴. It is contemplated that the antisense oligonucleotides may be packaged in a lipid vesicle before "shotgun" delivery of the antisense oligonucleotide.

Frequently, it will be desirable or necessary to introduce the pharmaceutical composition to the brain, either directly or indirectly. Direct techniques usually involve placement of a drug delivery catheter into the host's ventricular system to bypass the blood-brain barrier. One such implantable delivery system used for the transport of biological factors to specific anatomical regions of the body is described in U.S. Patent 5,011,472²⁵ which is herein incorporated by reference.

Indirect techniques, which are generally preferred, usually involve formulating the compositions to provide for drug latentiation by the conversion of hydrophilic drugs into lipid-soluble drugs. Latentiation is generally achieved through blocking of the hydroxy, carbonyl, sulfate, and primary amine groups present on the drug to render the drug more lipid soluble and amenable to transportation across the blood-brain barrier. Alternatively, the delivery of hydrophilic drugs may be enhanced by intra-arterial infusion of hypertonic solutions which can transiently open the blood-brain barrier.

Other suitable formulations for use in the present invention can be found in *Remington's Pharmaceutical Sciences*²⁶.

The antisense oligonucleotides or the pharmaceutical composition comprising the antisense oligonucleotides may be packaged into convenient kits providing the necessary materials packaged into suitable containers.

Utility

The antisense oligonucleotides of the present invention may be used for a variety of purposes. They may be used to inhibit the expression of the ribonucleotide reductase gene in a microorganism, resulting in the inhibition of growth of that microorganism. They may be used to inhibit the expression of the secA gene in a microorganism, resulting in the inhibition of growth of that microorganism. The oligonucleotides may be used as hybridization probes to detect the presence of the ribonucleotide reductase gene or the secA gene in the microorganism. When so used the oligonucleotides may be labeled with a suitable detectable group (a radioisotope, a ligand, another member of a specific binding pair, for example, biotin). The oligonucleotides may also be used to determine the presence of a particular

microorganism in a biological sample. Finally, the oligonucleotides may be used as molecular weight markers.

In order to further illustrate the present invention and advantages thereof, the following specific examples are given but are not meant to limit the scope of the claims
5 in any way.

EXAMPLES

In the examples below, all temperatures are in degrees Celsius (unless otherwise indicated) and all percentages are weight percentages (also unless otherwise indicated).

10 In the examples below, the following abbreviations have the following meanings. If an abbreviation is not defined, it has its generally accepted meaning:

	μM	=	micromolar
	mM	=	millimolar
15	M	=	molar
	ml	=	milliliter
	μl	=	microliter
	mg	=	milligram
	μg	=	microgram
20	IPTG	=	isopropyl- β -D-thiogalactoside
	PAGE	=	polyacrylamide gel electrophoresis
	PVDF	=	polyvinylidene difluoride
	rpm	=	revolutions per minute
	OD	=	optical density
25	CFU	=	colony forming units
	ΔG	=	free energy, a measurement of oligonucleotide duplex stability
	kcal	=	kilocalories

General Methods in Molecular Biology:

Standard molecular biology techniques known in the art and not specifically described were generally followed as in Sambrook et al.¹⁸; Ausubel et al.¹⁹; and Perbal²⁷.

- 5 The antisense oligonucleotides in Tables 1, 2 and 3 were selected from the sequence complementary to the ribonucleotide reductase or secA genes of *E. coli* such that the sequence exhibited the least likelihood of showing one or more of duplex formation, hair-pin formation, and homooligomer/sequence repeats but had a high to moderate potential to bind to the ribonucleotide reductase gene or the secA gene
- 10 sequence. These properties were determined using the computer modeling program OLIGO Primer Analysis Software, Version 5.0 (distributed by National Biosciences, Inc., Plymouth, MN).

- The antisense oligonucleotides in Table 4 were selected on the basis that the sequence is highly conserved for the secA genes between two or more microbial
- 15 species. This property was determined using the BLASTN program (Altschul, et al.¹⁶) of the University of Wisconsin Computer group (GCG) software (Devereux J. et al.¹⁷) with the National Center for Biotechnology Information (NCBI) databases

- Phosphorothioate oligonucleotides comprising the desired sequences were specially ordered either from Boston BioSystems, Bedford MA; Canadian Life
- 20 Technologies, Burlington, Canada; Dalton Chemical Laboratories, Inc., North York, Canada; Hybridon, Inc., Milford Ma; Oligos Etc., or Oligos Therapeutics, Inc., Wilsonville OR; or TriLink Bio Technologies, San Diego, CA. Antisense oligonucleotides may also be made by methods known in the art.

- Polymerase chain reaction (PCR) was carried out generally as in PCR
- 25 *Protocols: A Guide To Methods And Applications*²⁸.

Example 1: Inhibition of mouse ribonucleotide reductase small subunit (R2) expression in *Escherichia coli* by antisense oligonucleotide AS-II-626-20

Competent BL21 (DE3) cells carrying a plasmid containing the mouse ribonucleotide reductase R2 gene were used. (Mann et al.³⁴) The antisense oligonucleotide, AS-II-626-20, GGCTAAATCGCTCCACCAAG [SEQ ID NO:266] is specifically complementary to the mouse ribonucleotide reductase R2 gene.

Approximately 10^{10} bacteria/ml were electroporated using a Cell Porator (Gibco BRL, Burlington, Canada) in micro electro-chambers (0.4 cm between the electrodes) at a pulse of 2.4 kV, 4 k Ω with either 20 μ M or 200 μ M of antisense oligonucleotide AS-II-626-20, following methods described by the manufacturer (Dower W.J.²⁹; Neuman et; and Taketo, A.³¹). Control populations were subjected to electroporation but without the antisense oligonucleotide AS-II-626-20.

The bacterial cells were then transferred to Luria-Bertani broth (Miller J.H.³²) containing 50 μ g/ml of ampicillin and 0.4 mM of isopropyl β -D-thiogalactoside (IPTG) (expression inducer) (Horwitz J.P.³³) to grow at 30°C on a shaker at 250 rotations per minute (rpm) for 5 hours.

The cells were harvested by centrifugation and treated with 2 x sample loading buffer (100 mM Tris[hydroxymethyl]aminomethane, pH 6.8, 200 mM dithiothrietol, 4% sodium dodecyl sulfate, 20% glycerol and 0.015% bromophenol blue) and sonicated (Olsvik, et al.³⁵) for 15 seconds. The supernatants were resolved by polyacrylamide gel electrophoresis (PAGE) (Laemmli U.K.³⁶).

The ribonucleotide reductase R2 expression was detected by Western blot. The protein gel was blotted onto polyvinylidene difluoride (PVDF) protein sequencing membrane. (Choy et al.³⁷). The presence of the mouse ribonucleotide reductase was detected with a rabbit anti-mouse R2 subunit antibody (Chan et al.³⁹). The presence of the antibody bound to the ribonucleotide reductase was detected using a second goat anti-rabbit immunoglobulin linked with horseradish peroxidase (Amersham Life Sciences, Oakville Canada).

The upper panel of Figure 14 is a photograph of the Western Blot results. The lower panel of Figure 14 is a photograph of the membrane stained with India ink to indicate the level of protein loaded in each lane.

It is clear that administration of either 20 μ M or 200 μ M AS-II-626-20 resulted in a marked reduction of mouse ribonucleotide reductase gene expression in the *E. coli* cells.

Example 2: Inhibition of bacteria *Escherichia coli* K12 growth by antisense oligonucleotides ER1-169 and ER2-724 targeting *E. coli* ribonucleotide reductase large subunit (R1) and small subunit (R2)

E. coli cells were electroporated by the method set forth in Example 1 with ER1-169 [SEQ ID NO:22] or ER2-724 [SEQ ID NO:145] at the concentrations shown in Figure 15, while the control cells received oligonucleotide AS-II-626-20 [SEQ ID NO:] (targeting mouse ribonucleotide reductase small subunit).

The *E. coli* cells were then transferred to fresh Luria-Bertani broth (Miller J.H.³²) to grow at 30°C on a shaker at 250 rpm for 3 hours. The flasks for the test and the control each contained the same number of bacteria per ml at the start of the experiment. The optical density at 590 nm (OD₅₉₀) of the cultures was measured at the start and at the end of the 3 hours. The inhibition of *E. coli* growth was calculated by comparing the increase in OD₅₉₀ values at the start and the end of the 3 hours of the oligonucleotide-treated cultures to the increase of the control cultures at the start and at the end of the 3 hours. (Carpentier P.L.⁴⁰)

The results indicate that ER1-169 [SEQ ID NO:22] and ER2-724 [SEQ ID NO:145] inhibited the growth of *E. coli*.

Example 3: Killing of *Escherichia coli* K12 by antisense oligonucleotides targeting the ribonucleotide reductase large subunit (R1) or the small subunit (R2)

E. coli cells (approximately 2×10^9) were incubated with 20 μ M of each of the phosphorothioate oligonucleotides set forth in Figure 12 on ice for 45 minutes. A

control without oligonucleotides was also incubated for each experiment. Cells were heat shocked by placing them in a 42°C bath for 45 seconds. (Sambrook J. et al.¹⁸)

Luria-Bertani (LB) broth (Miller J.H.³²) was added and the samples were incubated at room temperature for 30 minutes. Dilutions of treated and untreated bacteria were incubated overnight at 37°C on culture plates containing LB medium, and the number of colonies was counted.

The number of killed bacteria was calculated by subtracting the surviving colony forming units (CFU/ml) of the oligonucleotide-treated bacteria from the CFU/ml of the control. Figure 16 shows the number of bacteria killed by treatment with the antisense sequences: ER1-640 [SEQ ID NO:43]; ER1-1059 [SEQ ID NO:62]; ER1-1320 [SEQ ID NO:75]; ER1-1315 [SEQ ID NO:74]; ER1-1326 [SEQ ID NO:76]; ER2-704 [SEQ ID NO:143] and ER2-983 [SEQ ID NO:152].

The results from Figure 16 show that antisense oligonucleotides complementary to either the R1 or R2 subunit of ribonucleotide reductase are effective as anti-bacterial agents.

Example 4: Inhibition of the secA protein expression in Escherichia coli following treatment with antisense phosphorothioate oligonucleotides

E. coli cells were heat shock transformed by the method set forth in Example 3 above with the 80 µM of each of the antisense phosphorothioate oligonucleotides set forth in Figure 17.

Luria-Bertani broth was then added to the treated *E. coli* cells and they were allowed to grow at 30°C on a shaker at 250 rpm for 3 hours.

Approximately the same quantity of treated and untreated bacteria, based on optical density, were washed in phosphate buffered saline, suspended in 2X Laemmli sample buffer (Laemmli U.K.³⁶), heated for 5 minutes at 95°C and subjected to SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis).

The gel was blotted onto polyvinylidene difluoride protein sequencing membrane by the methods set forth in Example 1. A rabbit polyclonal SecA antiserum (der Blaauwen et al.⁶) was used to detect the expression of the *E. coli* secA gene. The presence of bound rabbit antibody was detected using a goat anti-rabbit immunoglobulin (Amersham Life Sciences, Oakville, Canada).

Figure 17 is a photograph of the Western Blot of *E. coli* cells treated with oligonucleotides ES799 [SEQ ID NO:195] (lane 1); ES1845 [SEQ ID NO:235] (lane 2); and the control (lane 3). When compared to the control, lane 3, the ES799 [SEQ ID NO:195] and ES1845 [SEQ ID NO:235] oligonucleotides clearly decreased the SecA protein levels in the treated *E. coli* cells. The top band in the Figure 17 represents SecA. Non-specific background bands appear below the SecA protein band.

It has been found that the antisense oligonucleotides are effective inhibitors of SecA expression in *E. coli*.

15 Example 5: Killing of Escherichia coli K12 by antisense secA oligonucleotides

E. coli cells were heat shock transformed by the method described in Example 3 above with either 100 μ M or 20 μ M of the antisense phosphorothioate oligonucleotides set forth in Figures 18a and 18b

Luria-Bertani (LB) broth (Miller J.H.³²) was added and the bacterial samples were incubated at room temperature for 30 minutes. Dilutions of treated and untreated bacteria were incubated overnight at 37°C on culture plates containing LB medium, and the number of colonies was counted.

The number of killed bacteria was calculated by subtracting the surviving colony forming units (CFU/ml) of the oligonucleotide-treated bacteria from the CFU/ml of the control. Figures 18a and 18b show the number of bacteria killed by treatment with the various antisense sequences. Accordingly, antisense oligonucleotides complementary to the secA gene act to inhibit the growth of *E. coli*.

Example 6: Effect of antisense oligonucleotides on Escherichia coli K12 growth

E. coli cells were heat shock transformed by the method described in Example 3 with either 16 μ M, 20 μ M or 80 μ M of each of the antisense phosphorothioate oligonucleotides set forth in Figures 19a-g.

5 Equal numbers of the treated *E. coli* cells were then transferred to flasks containing fresh Luria-Bertani broth to grow at 30°C on a shaker at 250 rpm. The number of bacteria per flask was determined by the turbidity of the cultures at OD₆₂₀ taken each hour (Carpentier P.L.⁴⁰).

10 Figures 19a-g show the rate of growth of the *E. coli* in each of the flasks after treatment with the various oligonucleotides. When growth curves of the treated and untreated cultures were statistically analyzed, the growth of the antisense treated cultures was found to be significantly inhibited when compared to the control cultures. The statistical p values are found in the Figures.

Claims:

1. An antisense oligonucleotide which is nuclease resistant and comprises from about 3 to about 50 nucleotides, which nucleotides are complementary to the ribonucleotide reductase gene or the secA gene of a microorganism.
- 5 2. The oligonucleotide of Claim 1 comprising one or more phosphorothioate internucleotide linkages.
3. An antisense oligonucleotide comprising from about 3 to about 50
10 nucleotides which is capable of binding to the ribonucleotide reductase gene or the secA gene of a microorganism, wherein the oligonucleotide comprises all or part of a sequence selected from the group consisting of SEQ ID NO:22; SEQ ID NO:43; SEQ ID NO:62; SEQ ID NO:74; SEQ ID NO:75; SEQ ID NO:76; SEQ ID NO:143; SEQ ID NO:145; SEQ ID NO:152; SEQ ID NO:164; SEQ ID NO:176; SEQ ID NO:186;
15 SEQ ID NO:188; SEQ ID NO:189; SEQ ID NO:191; SEQ ID NO:192; SEQ ID NO:195; SEQ ID NO:197; SEQ ID NO:206; SEQ ID NO:212; SEQ ID NO:220; SEQ ID NO:229; SEQ ID NO:235; SEQ ID NO:254; SEQ ID NO:261; SEQ ID NO:262; SEQ ID NO:263; SEQ ID NO:264; and SEQ ID NO:265.
- 20 4. A pharmaceutical composition comprising a pharmaceutically acceptable excipient and an effective amount of an oligonucleotide which is nuclease resistant and comprises from about 3 to about 50 nucleotides, which nucleotides are complementary to the ribonucleotide reductase gene or the secA gene of a microorganism.
- 25 5. The pharmaceutical composition comprising a pharmaceutically acceptable excipient and an effective amount of an oligonucleotide comprising from about 3 to about 50 nucleotides which is capable of binding to the ribonucleotide reductase gene or the secA gene of a microorganism, wherein the oligonucleotide comprises all or part of a sequence selected from the group consisting of SEQ ID NO:22; SEQ ID NO:43;
30 SEQ ID NO:62; SEQ ID NO:74; SEQ ID NO:75; SEQ ID NO:76; SEQ ID NO:143;

SEQ ID NO:145; SEQ ID NO:152; SEQ ID NO:164; SEQ ID NO:176; SEQ ID
NO:186; SEQ ID NO:188; SEQ ID NO:189; SEQ ID NO:191; SEQ ID NO:192; SEQ
ID NO:195; SEQ ID NO:197; SEQ ID NO:206; SEQ ID NO:212; SEQ ID NO:220;
SEQ ID NO:229; SEQ ID NO:235; SEQ ID NO:254; SEQ ID NO:261; SEQ ID
5 NO:262; SEQ ID NO:263; SEQ ID NO:264; and SEQ ID NO:265.

6. A method of inhibiting the expression of a ribonucleotide reductase gene in
a microorganism having a ribonucleotide reductase gene, comprising administering to
said microorganism or to a cell infected with said microorganism an effective amount
10 of an antisense oligonucleotide comprising from at least about 3 nucleotides which are
complementary to the ribonucleotide reductase gene of the microorganism under
conditions such that the expression of the ribonucleotide reductase gene is inhibited.

7. The method according to Claim 6, wherein said microorganism is a bacterial
15 cell.

8. The method according to Claim 6, wherein said microorganism is a virus.

9. The method according to Claim 6 wherein the antisense oligonucleotide
20 comprises a sequence selected from the group consisting of SEQ ID NO:22; SEQ ID
NO:43; SEQ ID NO:62; SEQ ID NO:74; SEQ ID NO:75; SEQ ID NO:76; SEQ ID
NO:143; SEQ ID NO:145; and SEQ ID NO:152.

10. A method of inhibiting the expression of the secA gene in a microorganism
25 having a secA gene, comprising administering to said microorganism an effective
amount of an antisense oligonucleotide comprising from at least about 3 nucleotides
which are complementary to the secA gene of the microorganism under conditions such
that the secA gene is inhibited.

11. The method according to Claim 10, wherein said microorganism is a bacterial cell.

12. The method according to Claim 11 wherein the antisense oligonucleotide
5 comprises a sequence selected from the group consisting of SEQ ID NO:164; SEQ ID
NO:176; SEQ ID NO:186; SEQ ID NO:188; SEQ ID NO:189; SEQ ID NO:191; SEQ
ID NO:192; SEQ ID NO:195; SEQ ID NO:197; SEQ ID NO:206; SEQ ID NO:212;
SEQ ID NO:220; SEQ ID NO:229; SEQ ID NO:235; SEQ ID NO:254; SEQ ID
NO:261; SEQ ID NO:262; SEQ ID NO:263; SEQ ID NO:264; and SEQ ID NO:265.

10

13. A method of inhibiting the growth of a microorganism having a
ribonucleotide reductase gene or a secA gene, which method comprises identifying the
microorganism and administering to said microorganism an effective amount of an
antisense oligonucleotide comprising from at least about 3 nucleotides which are
15 complementary to either the ribonucleotide reductase gene or the secA gene of the
microorganism under conditions whereby the growth of the microorganism is inhibited.

14. The method according to Claim 13, wherein said microorganism is a
bacterial cell.

20

15. The method according to Claim 13, wherein said microorganism is a virus.

16. The method according to Claim 13 wherein the antisense oligonucleotide
comprises a sequence selected from the group consisting of SEQ ID NO:22; SEQ ID
25 NO:43; SEQ ID NO:62; SEQ ID NO:74; SEQ ID NO:75; SEQ ID NO:76; SEQ ID
NO:143; SEQ ID NO:145; SEQ ID NO:152; SEQ ID NO:164; SEQ ID NO:176; SEQ
ID NO:186; SEQ ID NO:188; SEQ ID NO:189; SEQ ID NO:191; SEQ ID NO:192;
SEQ ID NO:195; SEQ ID NO:197; SEQ ID NO:206; SEQ ID NO:212; SEQ ID
NO:220; SEQ ID NO:229; SEQ ID NO:235; SEQ ID NO:254; SEQ ID NO:261; SEQ
30 ID NO:262; SEQ ID NO:263; SEQ ID NO:264; and SEQ ID NO:265.

17. A method for treating a mammalian pathologic condition mediated by microorganisms, which method comprises identifying a mammal having a pathologic condition mediated by microorganisms having a ribonucleotide reductase gene or a secA gene and administering to said mammal an effective amount of an antisense
- 5 oligonucleotide comprising at least about 3 nucleotides which are complementary to either the ribonucleotide reductase gene or the secA gene of the microorganism under conditions such that the growth of the microorganism is inhibited.

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1 atgaatcaga atctgctggg gacaaagcgc gacggtagca cagagcgcat caatctcgac
61 aaaatccatc gegtctctgga ttgggcggca gaaggactgc ataacgtttc gatttcccag
121 gtcgagctgc gctcccacat tcagttttat gacggtatca agacctctga catccacgaa
181 accattatca aggtgcgc agacctgac tcccgtagg cgcggatta tcagtatctc
241 gccgcgcgc tggcgatctt ccacctgct gaaatggc gagatggca aatcagataa tcactgctg
301 gcgctgtacg accacgtggg gaaatggc gttcaagcag atggacacct ttatcgatca cgaccgtgat
361 gaagactaca cggagaaga gttcaagcag cgttaagcag ctggaaggca aatatctggt acagaaccgc
421 atgaccttct cttatgctgc aatcttatga ggcgcgccag ttcccttata ttctagttgc cgcgtgcttg
481 gtgaccggcg aatcttatga ggcgcgccag aacgcgcctg caatatgtga agcgttttta cgcgcgggtt
541 ttctcgaaat accgcgctga aacgcgcctg gccgcgcga atcatgtccg gcgtgcgtac cccgactcgt
601 tccacattta aatttctgt aatcttatga ggcgcgccag ggtgacagcc tggattccat caacgccacc
661 cagttcagct cctgcgtact gtcgagtg cgttcccag cgtgcccagg tggcatcaa cgcgggct
721 tccagcgca ttgttaata cgttcccag ggtgacagcc tggcatcaa cgcgggct
781 attcgtgcgc tgggtagccc gattcgcgt tctgctctc agggcgggtg gcgcggcggg
841 ttctacaaac atttccagac agcggtgaa tctgctctc agggcgggtg gcgcggcggg
901 gcggcaacgc tgttctacc gatgtggcat ctggaagtgg aagccctgct ggtgttgaaa
961 aacaaccgtg gtgtggaagg caaccgcgtg cgtcatatgg actacggggt acaaatcaac
1021 aaactgatgt ataccgtct gctgaagggt gaagatatca cctgttccag cccgtccgac
1081 gtaccggggc tgaacgacg gttcttcgc gatcagggaag agtttgaaag tctgtatacc
1141 aaatatgaga aagacgacag catccgcaag cagcgtgtga aagccgttga gctgttctcg
1201 ctgatgatgc aggaacgtgc gttaccggt cgtatctata ttcaagaactg tgaccactgc
1261 aatacccata gcccgtttga tccggccatc gcgcagtgcc gtcagttctaa cctgtgcctg
1321 gagatagccc tgcgcgacaa accgctgaac gacgtcaacg acgagaacgg tgaatcggc

FIG. 1A

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1381 ctgtgtacgc tgtctgcttt caacctgggc gcaatttaata acctggaatga acctggaagag
 1441 ctggcaattc tggcggttcg tgcacttgac gcgctgctgg attatcagga ttaccggatc
 1501 ccggccgcca aacgtggagc gatgggtcgt cgtacgctgg gtattggtgt gateaacttc
 1561 gcttactacc tggcgaaacga cggtaaacgc tactccgacg gcagcgccaa caacctgacg
 1621 cataaaacct tcgaagccat tcagtatcac ctgctgaaag cctctaata gctggcgaaa
 1681 gagcaaggcg cgtgcccglt gtttaacgaa accacttacg cgaagggat cctgccgac
 1741 gataccata agaaagatct ggataccatc gctaattgagc cgtgcattta cgaactgggaa
 1801 gctctgcgtg agtcaatcaa aacgcacgggt ctgcgtaact ccacgctttc tgctctgatg
 1861 ccgtccgaga cttcttcgca gatctctaac gccactaacg gtattgaacc gccgcgcggt
 1921 tacgtcagca tcadagcgtc gaaagacgggt attttgcgcc aggtgggtgcc ggactacgag
 1981 cacctgcacg acgectatga gctgctgtgg gaaatgccgg gtaacgatgg ttatctgcaa
 2041 ctggtgggta tcotgcagaa atttatcgat cagtcgatct ctgcccaaac caactacgat
 2101 ccgtcacgct tcccgtcagg aaaagtgcg atgcagcagt tgetgaaaga cctgctcacc
 2161 gcctacaaat tcgggggtcaa aacactgtat tatcagaaca cccgtgacgg cgctgaagac
 2221 gcacaagacg atctgggtgcc gtcaatccag gacgatggct gcgaaagcgg cgcattgtaag
 2281 atctga

FIG. 1B

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7381 ctggtgccgt caatccagga cgtggctgc gaaagcggcg catgtaagat ctgatatgtga
7441 gatgccggat gcggcgtaaa cgccttatac gccctacggc tcggtttgtgta ggcctgatata
7501 gacgcgccag cgtcgcatca ggtccgggt gccggatgca gcgtgaacgc cttatccggc
7561 ctacggctcg gatltgtagg cctgataaga cgcgccagcg tgcctcagg cacaggatgc
7621 ggcgtaaaat gccctatccg gcattaaact cccaacagga cacactcatg gcataatacca
7681 ccttttcaaa gacgaaaaat gatcagctca aagaaccgat gttctttggt cagccggtca
7741 acgtggctcg ctacgatcag caaaaatatg acatcttcga aaagctgac gaaaagcagc
7801 tctctttctt ctggcgctcg gaagaagtgt acgtctcccg cgaccgtata gattaccagg
7861 cgtgccgga gcacgaaaaa cacatcttta tcagcaacct gaaatatcag acgtgctg
7921 attccattca ggtcgtagc cggaaegtgg cgtattgcc gcttatttct attccggaac
7981 tggaaacctg ggtcgaaacc tgggcgttct cagaaacgat tcattcccg tcctatactc
8041 atatcattcg taatctcgtt aacgatccgt ctgttgtgtt tgacgatata gtcaccaacg
8101 agcagatcca gaaacgtgcg gaagggatct ccagctatta cgtgagctg atcgaaatga
8161 ccagctactg gcactctgctg ggcgaaggta cccacaccgt taacggtaaa actgtgaccg
8221 ttacccctgcg cgagctgaag aaaaaactgt atctctgccct gatgagcgtt aacgcgctgg
8281 aagcgattcg tttctacgtc agctttgctt gttcccttcgc atttgcagaa cgcgaattga
8341 tgggaaggcaa cgccaaaatt attcgctga ttgcccgcga cgaagccctg cacctgaccg

FIG. 2A

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8401 gcacccagca tatgtgaat ctgtgcga gcggcgcgga cgatcctgag atggcggaaa
8461 ttgccgaaga gtgtaagcag gagtctatg acctgtttgt tcaggcagct caacaggaga
8521 aagactgggc ggattatctg ttccgcgacg gttcgatgat tggctgaaat aaagacattc
8581 tctgccagta cgttgaatac atcaccataa tccgtatgca ggcagtcggt ttggatctgc
8641 cgttccagac gcgtccaac ccgatcccgt ggatcaaac ttggtggtg tctgataacg
8701 tgcaggttgc tccgcaggaa gtggaagtea gtctctatct ggtcgggcag attgactcgg
8761 aagtggacac cgacgatttg agtaacttcc agctctgatg gccgcggtta cctgcgcgat
8821 cactggcaca caactgctgt gccaggatga acaccttcc cttctggcgg cgtgggaatc
8881 ccacaatgtg gcggttgagt accagtgtcg cgaaggttac tgcggtcct gtcgcacacg

FIG. 2B

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301 gtgaacgtcg atctggtgcc ggaatgcagcg gatacgcctcc gggcgcaagg atttcgtcaa
361 ttaccggtgg tgatggcggg cgatttgagc tggctctggt tccgcccgga catgattaac
421 cgtctgcacc cgacacccca cgcggcaaac gcatgagcgc gctcgtctac ttctccagca
481 gctctgaaaa tacgcaccgc tttatgcagc gtctggggtt gcctgccacg cgtattccgc
541 tcaatgagcg ggagcgaaat caggtagacg aaccgtacat tctggttgct ccgtcatacg
601 gcggcgggcg gatggccggt gcggtgccgc gacaggtgat ccgctttta aatgatgaac
661 acaaccgggc gcgcattcgc ggcgttatcg cctccggtaa tcgcaatttc ggcgatgcct
721 ggggatgcgc tggcgatgtg atagcacaaa aatgcggcgt cccctggctg taccgctttg
781 agctcatggg cacacaacgc gacatcgata atgtccgaaa aggagtaaat gaatttlggc
841 acaactacc cgggagcgcg taatgcagga aacctggat taccacgccc tgaacgcgat
901 gctgaatttt tacgataaag caggccatat tcagttcgac aaggaccagc aggcgatcga
961 cgccttcttt gccacccacg tccgcccgca ttccgtgacg tttgccagcc agcatgaacg
1021 tctggggacg ctggttcggg aagggtatta cgatgacgcc gtctcgcgc gttacgaccg
1081 cgccttcgtc cttcgacctgt tcgagcacgc caatgccagc ggtttcgtt tccagacgtt
1141 tcttgccgc tggaaattct ataccagtta caccgtgaaa accttcgacg gcaaacgtta
1201 tctggaaacac tttagagatc ggtgacaat ggtggcgttg acgctggcgc aggtgacga
1261 aacgctggcc acccaactga ccgatgaaat gctttctggt cgtttcagc ccgtacccc
1321 gactttttta aattgcggca aacagcagcg tggggaaactg gtctcctgct tctgtctccg
1381 tatcgaagac aacatggagt cgatcgggcg ggcggtgaat tcggcgctgc aactctccaa
1441 acgcggcggc ggcgtcgcgt tttactctc caatctgcgc gaggcgggag cgcgatcaa
1501 acgcattgag aatcagcttt ccggcgtgat cccggtgatg aaaaatgctgg aagacgcgtt
1561 ttcgtatgcc aaccaacttg gcgcgcgcca gggggccggc gcggtttatc tccatgcga
1621 ccataccgat attctgcgtt ttctggatoc caaacgagaa aacgctgacg aaaaaatccg

FIG. 3A

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1681 gatcaaaacg ctctctctcg gcggtgtgat cccggatata accttcggc tggcgaaaga
 1741 aaacgcgcaa atggcgctct tttcgcccta tgacatacaa cgacgctacg geaaaccgtt
 1801 tggcgatata gccattagcg aacggtagca tgaatttaatt gccgatccgc acgtgcgcaa
 1861 aacctatatt aacgcccgtg acttttttca aacactggcg gagattcagt tcgaatccgg
 1921 gtatccctac atcatgtttg aagatacgggt aaaccgcgcg aatcccattg ctggtcgcat
 1981 taatatgagc aacctgtget cagaaatttt acagggtcaat agcgettccc gttacgacga
 2041 taaccttgac tatcccaca tegggcattga catctectgc aatctcggct cgtgaatat
 2101 cgctcaagtc atggattcac cggacattgg ccgtaccgta gaaaccgcta ttcgggcct
 2161 gacggcggtg tgggacatga gccatatatcg cagcgtgccc tcaatagccg ccgtaatatgc
 2221 cgctctcat gccatcggtc tgggccagat gaatctgcat ggctatctgg cgagggaagg
 2281 tattgacctac ggttcgccgg aggcgttggg tttcaccat ctctatttt acaccattac
 2341 ctggcatgcc gtgcatactt caatgcggct agcccgcgaa cgcggaacaa ccttcgccgg
 2401 atttgcgcag tcgcgtatg ccagcggcga ctattttacg cagtatttac aggacgactg
 2461 gcaaccgaaa acagcgaaag tcagggcgtt atttgccgc agcgcatcta cgtgcccac
 2521 acgagaaatg tggctaagc tgcgcgacga tgtgatgcgc tatggcatct ataaccacaa
 2581 tttgcaggcg gtgccgccga ccggttcgat ttcttacctt aatcatgca cctccagcat
 2641 tcatccgatt gtggccacaa ttgagattcg caaagagggc aaaccgggc gtgtgtatta
 2701 cccgcgcggc ttatatgaca atgaaaacct ggacatgtat caggatgctt acgatatcgg
 2761 tccggaaaaa attattgata cctatgccga ggccacgcgc cagtcgac aagggtgtc
 2821 gctcaccctg tttttcccg ataccgccac gaccgcgat atcaacaagg cgcagatcta
 2881 tgcctggcga aaaggtatta agtccctgta ttacatccgg cttcgccagt tggcgctgga
 2941 aggtactgaa attgaagget gcgtatcctg cgcgctataa ggaagccat atgaatttat
 3001 ctcgtattag cgccatcaac tggaaacaaga tccaggacga caaagatctg gaggtatgga

FIG. 3B

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3061 accggctgac cagtaacttc tggctgccgg aaaaagtgc gttatcgaaat gatattccgg
3121 cctggcagac gctgagcgcc gccgaacagc agctcaccat tcgcgtgttt acgggactta
3181 cgtgctcga cactatccag aacatcgacg gcgcgccgtc gttaatggca gatgccatca
3241 cgcgcgatga agaggcagtg ctgtcgaaac tcagctttat ggaagcggta cacgcccgt
3301 cttacagttc tattttctcc acgctgtgcc agcggaaaga ggttgatgcc gctacgcct
3361 ggagcgaaga aaacccaccg cttcagcgta aggcgcagat tattttagct cattacgtca
3421 gcgatgaacc gctaaagaaa aagattgcca gcgtcttttt agagtccttt ctgtcttatt
3481 cggcttctg gttgccgatg tatttctcca gccgcggtaa gctcacgaac actgccgacc
3541 tgattcgttt aatcattcgc gatgaagcgg ttcaacggta ttatatggc tataagtatc
3601 agatagcgct acaaaaacta tcggcaatcg agcgtgaaga gttaaagctt ttccgcgtgg
3661 atttgttgat ggaactgtac gacaacgaaa tccgtacac agaagcgtta tatgcggaaa
3721 cggctgggt taacgacgc taagccttct tgtgtacaa cgccaataaa gccttaatga
3781 acctgggtta tgaggcgtta ttccgcggc agatggcaga cgtgaatccc gcaatccttg
3841 ccgcgtctc gccgaatgcc gacgaaacc atgatttctt ttccggctca ggttcactct
3901 atgtgatggg gaaacacglt gaaacccaag ccgaagactg gaatttttaa ccttacgggc
3961 atgggaaata acgttacatt tcccatgctt ttatttcaag caatagggag tcaaatcgcg
4021 caaatattac aacatgtctt acactcaata cgagtgaat tattcacctg gattccccca
4081 attcagggtg atttttgtg gttgttccaa aaaatatctc ttccctccca ttccgcgttca
4141 gcccttatat catgggaaat cacagccgat agcacctcgc aatatctatg ccagaagcaa
4201 attcagggtt gtctcagatt ctgagtatgt tagggtagaa aaaggtaact atttctatca
4261 ggtaacatat cgacataagt aaataacagg aatcattcta ttgcattgga attaattag
4321 aagtgaagaa tctgtataaa atatttgag agcatccgca gcgtgccttc aatatattg
4381 aaaagggact atcgaagag caaatactgg aaaaaacggg gctatcgctt ggcgttaag

FIG. 3C

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4441 acgccagtct ggcattgaa gaaggcgaga tatttgatcat catgggatta tccggctcgg
4501 gtaaatccac aatggtagc cttctcaatc gctgattga acccaccgcg ggcacaggtac
4561 tgattgacgg cgttgatatt gccaaaatat cagacgctga gcttcgcgag gtgcgcagga
4621 aaaagattgc gatggtcttc cagtcatttg cgtcatgccc gcataatgacc gtgctggata
4681 atacggcatt cggatatgaa ttagcgggca tcgcggcgca agagcgtcgc gaaaaagcgc
4741 tggacgcctt gcgtcagggtg gggcttgaga attacgctca cgcctacccg gatgaacttt
4801 ccggtgggat gcgtcagcgt gttgggcttg cccgcgcgct ggcaatcaac cctgatatac
4861 tattaatgga tgaagcgltt tccgcccctcg atcc

FIG. 3D

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1 gaattcttat ttccctagc ttggattta ttctacttc ctatgatctt ttattctcga
61 ttattatttt tgccttggca attattatca tttttcgaca taaacaaaac ctcaaaagaa
121 tcaaaaatca ttgtgaatcc cttgtccctt ttggttttaa cttatcgaga caaaagaaa
181 aatagcaca tatattgtgt tgtttttctt tttttacata atttaacct atattctaga
241 tctttaattt gactagatat tttttttacg ctaataaga ctataaaaac tcgagaaaaa
301 gtaaggact ttttactccc gtcataaaaa tatattggcc caaaggaga tttaaaatgg
361 ttacagttta ttctaaaaac aattgtatgc aatgcaaaat ggtcaaaaaa tggctttctg
421 aacacgaat tgcatttaac gaaatcaata ttgatgaaca gcctgaattt gtcgaaaaag
481 taattgaaat gggttttcga gctgctcctg taatcacaaa agatgatttc gccttttctg
541 gttccgtcc ttctgaatta gcaagttgg cttaatatga aacttgctta ttccagtgtg
601 actggacaaa cgcgtcgltt tgtttctaaa acagactgc cgaatgtcga aattacacct
661 gacgatgatt tagagatgga cgagccttcc cttttgataa ctccctctta tgetgaagaa
721 tcaccaaccg tttctaaatc aatagacgtt atggactcgg tttttgactt tatggcttat
781 aatgataatt ataaccattg tcgtggaatt atcggcaactg gaaatcgtaa ttttgctggc
841 atctatatatt ttaccgctaa agaagtttca gcaaaatatac aaattccact ttatatgat
901 tttgagttta atggtacgcc agctgatgtt gctgctgttg aaaaactcgc tgcacagctt
961 gatcaaggag cgaagtcac ctttaaaat ccgctgtgat tttttatggc ttcacctat
1021 ttgagtgaag ctt

FIG. 4

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1  cagctgtact ggcataacga catttatact gtcgtataaa attcgactgg
51  caaatctggc actctctccg gccagggtgaa ccagtcgttt ttttttgaat
101 tttataagag ctataaaaaa cggtgccgaac gctgttttct taagcacttt
151 tcegcacaaac ttatcttcat tcgtgctgtg gactgcagge tttaatgata
201 agatttgtgc gctaaatacg tttgaatatg atcgggatgg caataacgtg
251 agtggataac tgacgecgtg ggcacagttt ggtaaacgct acttctggcc
301 gcatacttta ttagggtggg ttgcggcgag tttagggttg cetgcgctca
351 gcaacgccgc cgaaccaaaac gcgcccgcaa aagcgacaa cgcgaaccac
401 gagccttcag ccaaggttaa ctltgggtcaa ttggccttgc tgggaagcgaa
451 cacacgccgc cgaattcga actattccgt tgattactgg catcaacatg
501 caattcgca cggtaatccgt catctttctt tcgcaatggc accgcaaaaa
551 ctgcccggtg ctgaagaatc ttltgcctctt caggcgcaac atcttgcatt
601 actggatacg ctacgcgcgc tgcgtaccca ggaaggcagc ccgtctgaaa
651 agggttatcg cattgattat gcgcatttta ccccaacagc aaaaattcagc
701 acgcccgtct ggataagcca ggcgcgaaggc atccgtgctg gccctcaacg
751 cctcacctaa caacaataaa cctttaactt atttatttaa ctccgcgaacg
801 cggggcggtt gagattttat tatgctaalc aatttgttaa ctaaggtttt
851 cggtagtcgt aacgatcgca cctgcgcgcg gatgcgcgaa gtggtcaaca
901 tcatcaatgc catggaaccg gagatggaaa aactctccga cgaagaaatg
951 aaggagaaaa ccgcagagtt tcgtcacagt ctggaaaaaa qcgaaatgct
1001 gaaaaatctg atcccggag ctttcgcagt ggtacgtgag gcaagtaagc
1051 gcgtcttttg tatgcgtcac ttcgacgttc agttactcgg cggatatggtt
1101 cttaacgaa cgtgcacgc cgaatgcat accggtgaaq gaaaaacccct

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FIG. 5A

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1151 gaccgcgaacc ctccctcctt acctgaacgc actaaccggt aaagcgctgc
1201 acgtagttaac cgtcaacgac tacctggcgc aacgtgacgc cgaatacaac
1251 cgtccgctgt ttgaattcct tggcctgact gtcggatata acctgccggg
1301 catgccagca ccggcaaacg cgaagctta cgcagctgac atcaattaca
1351 gtaccaacaa cgaatacggc tttagactacc tgcgcgacaa catggcgttc
1401 agccctgaag aacgtgtaca gcgtaaactg cactatgcgc tggtagacga
1451 agtgaactec atcctgactg atgaagcgcg tacaccgctg atcatttccg
1501 qcccggcaga agacagctcg gaaatgtata aacgcgtgaa taaatattt
1551 ccgcacctga tccgtcagga aaagaagac tccgaacct tccagggcga
1601 aggecacttc tcggtggacg aaaaatctcg ccaggtgaac ctgaccgaac
1651 gtggtctggt actgattgaa gaactgctg tgaagagagg catcatggt
1701 gaaggggagt ctctgtactc tccggccaac atcatgctga tgcaccacgt
1751 aacggcggcg ctgcgcctc atgcctgtt taccctgac gtcgaactaca
1801 tcgttaaga tggtaggtt atcctcgttg acgaacacac cagtcgtacc
1851 atgcaggacc gtcctggtc cgtggtctg caccaggtg tgaagcgaa
1901 agaaagtgtg caqatccaga acgaatacca aacgtggtt tcatcacct
1951 tccagaaacta ctccgtctg tatgaataac tggcggggt gaccggtact
2001 actgataccg aagctttcga atttagctca atctacaagc tggataccgt
2051 cgttgattccg accaacctc caatgattcg taaggtctg ccggaccctg
2101 tctacatgac tgaagcgga aaaaatcagg cgaatcatga agatatacaa
2151 gaacgtactg cgaagggcca gccgtgtctg gtgggtacta tctccatcga
2201 aaaatcggag ctggtgtcaa acgaactgac caagccggt attaagcaca
2251 acgtcctgaa cgccaattc cagccaacg aagcggcgt tgttgcctca

FIG. 5B

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2301 gcaggattatc cggctgcggt gactatcgcg occaatatgg cgggtcgtgg
2351 tacagatatt gtgctcggtg atagctggca ggcagaaagt gccgcgctgg
2401 aaatccgac cgcagagcaa attgaaaaa ttaagccga ctggcaggta
2451 cgtcacgatg cggtaactga agcaggtagc ctgcatalca tcggtaccga
2501 gcgtcacgaa tcccgtccta tcgataacca gttgcgcggt cgttctggtc
2551 gtcaagggga tcttgattct tcccgtttct acctgtcgat ggaagatgca
2601 ctggtgccta tttttgtctt cgaccgagta tccggcatga tgcgtaaact
2651 gggatatgaa ccaggcgaa ccatgaaaca cccgtgggtg actaaagcga
2701 ttgccaaagc ccagcgtaaa attgaaagcc gtaacttcga cttcgttaag
2751 caactgctgg aatatgatga cgtggctaac gatcagcgtc gcgccattta
2801 ctccacgagt aacgaactgt tggatgtcag cgatgtgagc gaaaccattta
2851 acagcatttc tgaagatgtg ttcaaaagca ccattgatgc ctacattcca
2901 ccacagtcgc tgaagaaat ataggatatt ccggggtgc aggaacgtct
2951 gaagaaacgt ttccaccctcg atttgccaat tgccgagtgg ctggataaag
3001 aaccagaaat gcatgaagag acgtgcgtg acgcatttct ggcgcagtcc
3051 atcgaaagtat atcaacgtaa agaaagaaatg attggtgctg agatgatgca
3101 tcaacttcgg aaggcgtca tctgcgaac gttgactcc ctgtggaaa
3151 agcacctggc agcgtggac tatctgcgtc aggatatacca cctgcgtggc
3201 tacgcacaga aagatccgaa gcagggaatac aacgtgaat cgttctccat
3251 gtttgacgca atgctggagt cgttgaata tgaagtatac agtacgctga
3301 gcaaaattca ggtacgtatg cctgaagagg ttgaggagct ggaacacacg
3351 cgtcgtatgg aagccgagcg tttagcgcaa atgcagcagc ttagccatca
3401 ggtatgacgac tctgcagcgg cagctgcact ggcggcgcaa accggagagc

FIG. 5C

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3451 gcacaaatagg acgtaacgat ccttgcccgt gcgattctgg taaaaatat
3501 aagcaatgcc atgcccgcct gcaataaaag ctaactgttg aagtaaaagg
3551 cgcaggattc tgcgcctttt ttataggttt aagacaatga aaaagctgca
3601 aattgcggta ggtattatlc gcaacgagaa caatgaaatc ttataacgc
3651 gtcgcgcagc agatgcgcac atggcgata aactggagtt tcccggcggg
3701 aaaattgaaa tgggtgaacc gccggaacag gcggtggtgc gtgaacttca
3751 ggaagaagtc gggattaccc ccaacattt ttcgctattt gaaaaactgg
3801 aatatgaatt c

FIG. 5D

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1 gatctacggc agaactcgtc gcttggagcg ttcgaccgac catctacctg
 51 ttcgacgtcg aactcgacca ctgaacgtaa tgcgcgccag cgcgaagtcct
 101 gtcagcgcgt ggagatcacc gcgcgtgggc gagggccgggt ggtgcgaggt
 151 gaggccctgcg ccgacagctt ctatgcccg cttgaatcag cgtcgtcaa
 201 actggagagc gtgcgcccg gtaaggatcg ccgcaagggtg cactacggcg
 251 acaaaacccc ggtttcgctg gccgaggcga ccgcggtggt gccagcgccg
 301 gagaacggct tcaacaccag accagccgag gcacacgac acgacgggtgc
 351 cgtcgtcgag cgggagcctg ggcggatcgt tcgcacccaa gaacaccccg
 401 ccaagccgat gtcggtcgat gacgcgtctt accagatgga gctggttggg
 451 cacgacttct tcttgttcta cgacaaggac accgaacggc cgtcgggtggt
 501 ctaccgccgg cagcctacg actacggctt gatccgtctg gcgtgatcgg
 551 cggcgccgcg cgctcgtaac ctaccatggg agtcgcctta tctaagact
 601 cctacacatg cggggacata gctgtgctgt cgaagtgtct gcgccttggc
 651 gaaggtcgca tggtaagcg cctcaagaa gttggcggact atgtcggcac
 701 tttgtccgac gatgtcgaga aactcaccga cgcgagctg agggcgaaaa
 751 ccgacgagtt caagcggcgg ctggccgacc agaaaaaccc agaaacccctc
 801 gacgacctgt tgcgcgagc cttcgccgtg gcccgcgagg ccgcctggcg
 851 ggtgctggac cagcggccgt tcgaactgca ggtgatgggt gcggccgccc
 901 tgcacctggg caacgttgc gagatgaaga ccggtgaagg caagacccctg
 951 acctgtgtgt tgccegtta cctcaatgca ctggccggca acggcgtgca
 1001 catcgtcacc gtaacgact acctggctaa acgcgacagt gagtggatgg
 1051 gccgcgtgca ccgcttcctc gggcttcagg tcgggggtgat ttgcgccacc
 1101 atgacacccg atgaacggcg ggtggcctat aacgcggaca tcacctacgg

FIG. 6A

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1151 caccataaac gagtttggt tegactacct ggcgacaaac atggcgcaact
1201 cactggatga tctggtgcag cgcgggcaac attacgccat tgtcgacgag
1251 gtcgattcca tectgatcga cgaaggccgc accccgctga tcatctccgg
1301 tcccgccgac ggcctccaac tggtaacacg agttcgccgg ttggcgccgc
1351 tgatggaaaa ggacgtccac tacgaggtcg atctacgca acgcaccgtc
1401 ggcgtgcacg agaagggtgt ggaattcgtc gaagaccagc tcggcatcga
1451 caacctgtac gaggcgccca actcgccgtt ggtcagctat ctaacaaacg
1501 ctctgaaggc caaagagctg ttacgcgcgc acaaggacta catcgctccgc
1551 gatggtgagg tgctcatcgt cgacgagttc accggccggg tgtgatcgg
1601 ccgcgcctac aacgagggca tcgaccaggc catcgaggcc aaggagcaacg
1651 tcgagatcaa ggccgagaa cagacgttg ccaccatcac gctgcagaac
1701 tacttccggc ttacgacaa gctcgccgc atgaccgga cgcgccagac
1751 ggaggcgcc gagctgcacg agatctacaa gctgggcgtg gtcagcatcc
1801 cgaccaacat gccgatgat cgtgaagacc agtccgacct gatctacaag
1851 accgaggagg ccaagtacat cgcggtggtc gacgacgtcg ccgagcgcta
1901 cgcgaaggga cagccggtgc tgatcggcac caccagcgtg gagcgctcgg
1951 agtatctgtc gcggcagttc accaagcggc gcataccgca caatgtgtc
2001 aacgcccaagt accacgagca agaggcgacc atcatcgcgg tggcgggccg
2051 ccgcggcggc gtcaccgtcg ccaccaacat ggccggtcgc ggcaccgaca
2101 ttgtgctggg cggcaacgtc gactttctca ccgatacagc gctgcgcgaa
2151 cggcctggat ccggtggaga cgcccgagga gtacgagggc gccctggcaat
2201 ccgaactgcc catcgtaaaa gaggaagcca gcaaggaggc caaggaaagta
2251 atcgaggccg gcggctgtac gtgctgggca ccgagcggcc acgagtcgcg

FIG. 6B

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2301 gcggtatcgac aaccagtgtgc gtggccgggtc cggccgcccag gggacccccgg
 2351 ggagtcgcgc ttctatttgt cgctgggtga cgagctgatg cgcgcttca
 2401 atggcgcgcc cttggagacc ttgttgacca ggtgaacct gcccgacgac
 2451 gtgccgatcg aagccaagt ggtcaccgg gccatcaaga gcgcccagac
 2501 ccaggctcag cagcagaact ttgaggctcg caagaacgtc ctcaatatcg
 2551 acgaggtgat gaaccagcag cgcaaggta tctacgccga gcgcgggcgc
 2601 atcctcgaag gcgaataact caaggaccag gcgctggaca tggctccgcga
 2651 tgtcatcacc gcctacgtcg acggcgcgac cggcggaagg tatgccgaag
 2701 attgggatct ggaecggttg tggacggcac tcaaacacct ctatccggag
 2751 gggatcaccc cggactcgct gacccgcaag gaccacgaat tcgagcgcgga
 2801 cgatctcacc cgcgaggagt tgctggagg actactcaag gacgccgaac
 2851 gtgcctatgc cgcacgggaa gccgaactcg aggaatcgc cggcgagggt
 2901 gcgatgcgcc agctggaacg caacgtgctg ctcaacgtca tagaccgtaa
 2951 gtggcgtgaa cacctctacg agatggacta cctcaaggag ggtatcgggc
 3001 tgcgcgcgat ggcgcacggc gatccgttgg tcgagtacca gcgtgagggc
 3051 tacgacatgt tcatggccat gctcgacggc atgaagaagg aatcgggtcgg
 3101 ctctctgttc aacgtcaccg tggaggcgggt ccccgccccg ccggttgccc
 3151 cggctgccga acccgcacag cttgccgaat tcgcccgcgc gcccgacgcc
 3201 gcgggcagca acgcagcgcg gtcgatggtg gcgcgcgcga aagagctcca
 3251 agtgcattac gcgccaaagg tgtgccagc gagtcgccc cttgacctta
 3301 ttccgggtccc gcggaggatg gctcgggtca ggtgcagcgc aacggcggtg
 3351 gagcccaaaa gacgccggcc ggagtgcgg ccggtgctag ccggcgcgag
 3401 cggcgcgaac gcgcccgccg acaaggcgcc ggcgccaagc cgccgaatc

FIG. 6C

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3451 ggtaagaag cgttagcgcg taggtgacg atgggtgtat cggtttctca
3501 gtteccagaa gtcaattccc ggcacacccc ggcacggcg cgcattgcaca
3551 tttegttgca cggcgggcaa ggggttcgt aatctacccc gttcgtcgac
3601 cttegtcggc gtcggtttctg ctggtagcgg ggttcggcgc ttctctggcg
3651 ttctctgact cgacaatcgt caacatcgcg ttcccgata tccagcgttc
3701 ctteccgtcc tacgacatcg ggagcctgtc ctggattctg aacggctata
3751 acatgctctt cgccgccttc atggttgagg cggcagggtt ggcgatttg
3801 ctgggcccga gacgacattc ctgtccggcg tctggtgtt caccattgcg
3851 tccgggctgt gcgcgctgc cggcagtgte gaggagtgg tggcgttcgg
3901 ggtgctgcag ggcacgggg ctgcatact cgtgcctcgt tcgctcgac
3951 tggtcgttga gggcttcgac cgggcggcgg cgcgacgct atcggcctgt
4001 ggggtgcggc ggcagcgatc cactagtctt agagcggcgc accgc

FIG. 6D

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1  tcaaacacca gaccagaagg aggcacaacg atcacggacg gtgccgttcg
51  tgcagcggga gcctggggcg gatcgttcgc accaagaac aacccggcca
101  cgccgatgtc ggtcgatgac gcgctctacc agatggagct ggttggacac
151  gacttcttct tgttctacga caaggacacc gaacggccgt cggtggtcta
201  ccgcgggcac gcctacgact acggttgat cegtetggcg tcatcggcgg
251  cgcgcgcgc gtcgtcact accatgggag tcgccttacc taagactcc
301  tacacatgcg gggacatagc tgtctgtcg aagtgtctgc gccctggcga
351  aggtcgcatt glcaagcgcc tcaagaaggc ggccgactat gtcggcactt
401  tgtccgacga tgcgagaaa ctacccgacg ccgagctgag ggcgaaaaac
451  gacgagttca agcaggctgg ccgaccagaa aaccccgacg accctcgacg
501  acctgttgc cgaggcctc accgtgccc gcgagaccgc cctgccgggt
551  gctggaccaa cgaccgttcg acgtgcaggt gatgggtacg accgccctgc
601  acctgggcga cgttgccgag atgtagaccg gtgaaggcaa gacctgacc
651  tgtgttttac ccgtttacct caatgccctg gccgccuacg gcgtgcacgt
701  agttaccgtc aacgactacc tgctaaacg cgacagtgag tggatggcc
751  gcgtgcaccg cttcctcggg cttcaggctg ggtgatattt ggcacccatg
801  aaccccgatg aacgccgggt ggcctataac gccgacatca cctacggcac
851  caataacgag ttgggttcg actacctgcg cgacaacatg gcgcactcac
901  tggatgatct ggtgcagcgc gggcaccatt acgccattgt cgacgaaggc
951  cgattccatc ctgatcgacg agggcggggc cccccccca tctccgccg

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FIG. 7A

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1001 gggcgccgc ctccaactgg ttcaaccgagt tcgccgggtt ggcgtgccgc
1051 ggctgggtttt ggacgtccac tacgaggtcg atctacgcaa acgcaccgtc
1101 ggcgtgcacg agaagggtgt ggaatttcgtc gaagaccagc tcggcatcga
1151 caacctgtac gagaccgcca actcgccgtt ggtcagctat ctcaacaacg
1201 ctctgaagc caagagctg ttcagccgcg acaaggacta catcgteccg
1251 gatggtgagg tgctcatcgt cgacgagttc accggccggg tgetgatcgg
1301 ccgccgtac aacgaggcca tgcaccaggc catcgaggcc aaggagcacg
1351 tcgagatcaa ggccgagAAC cagacgtgg ccaccatcac gctgcagaac
1401 tacttccggc tctaggagaa gctcgccggg atg

FIG. 7B

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1  tggcttgatt caaactagtg aacaataaat taagtttaaa gcacttgtgt
51  ttttgcacaa gtttttttat actccaaaag caaattatga ctatttcata
101 gttcgataat gtoatttgtt gaatgaacaa tagtgactat gctaattgta
151 atggatgtat atatttgaaat gttaagttaa taatagtatg tcagtcctatt
201 gtatagtcag agtcgaaaat cgtaaaatat ttatataata atttatagg
251 aagtataatt gcgtatttgg aatatattta ttagtgataa acttgttgac
301 aacagaatgt gaatgaagta tgtcataaat atatttatat tgattctaca
351 aatgagtaaa taagtataat tttctaacta taatlgataa gatataattgt
401 tglaggccaa acagtttttt agctaaagga gcgaacgaaa tgggattttt
451 atcaaaaatt cttgatgga atataaaga aatlaaacag ttagglaaac
501 ttgctgataa agtaatcgct ttgaagaaa aaacggcaat ttaactgat
551 gaagaaattc gtaataaac gaacaattc caacagaat tagctgacat
601 tgataatgtc aaaaagcaaa atgattattt acataaaatt ttaccagaag
651 catatgcact tgttagagaa ggctctaaac gtgtattcaa tatgacacca
701 tataaagttc aaattatggg tggatttgca attcataaag gtgatatacg
751 tgagatgaga acaggtgaag gtaaacattt aacagcgaca atgccaacat
801 acttaaatgc attagctggg agagggtgtc acgttattac agtcaatgaa
851 tacttatcaa gtgttcaag tgaagaaatg gctgagttat ataacttctt
901 aggtttgact gtcggattaa acttaaacag taagacgaca gaggoaaaaa
951 gtgaagcata cgcacaagac attacttaca glactaataa tgagctaggt
1001 tttgattact tacgagataa catggtgaat tattctgaag atagggtaat
1051 gcgtccatto cattttgcaa teattgatga ggtggactca attttaatcg

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FIG. 8A

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1101 acgaggcagc tacgccatta attatttctg gtgaagctga aaagtcacg
1151 tcactttata cacaagcaaa tgtttttgcg aaatgttaa acaggacga
1201 tgattataa tacgatgaa aacggaagc tgtacattta acagaacaag
1251 gtgcggtata agctgaacgt atgttcaaa ttgaaaactt atatgatgta
1301 caaatgttg atgttattag teatatcaac acagctttac gtgcgcacgt
1351 tacattacaa cgtgacgtag actatatggt tgttgatggc gaagtattaa
1401 ttgtcgatca atttacagga cgtacaatgc caggccgtcg ttctctcgaa
1451 ggtttacacc aagctattga agcgaaggaa ggcgttcaaa ttcaaaaatga
1501 atctaaaact atggcgctca ttacattcca aaactatttc agaattgtaca
1551 ataaacttgc gggtagtaca ggtacagcta aaactgaaga agaagaattt
1601 agaatattt ataacatgac agtaactcaa attccgacaa ataaccctgt
1651 gcaacgtaac gataagctcg atttaattta cattagecaa aaaggtaat
1701 ttgatgcagt agtagaagat gttgttgaaa aacacaagc agggcaacca
1751 gtgctattag gtactgttgc agttgagact tctgaatata ttcaaattt
1801 acttaaaaaa cgtggtatcc gtcattgatgt gttaatgcg aaaaatcatg
1851 aacgtgaagc tgaattggt gcaggcgctg gacaaaaag tgccgttact
1901 attgccacta acatggctgg tcggggtaca gatatacaat taggtgaagg
1951 cgtagaggaa ttaggcggtt tagcagtaat aggtacagag cgacatgaat
2001 ctcgtcgtat tgatgaccag ttacgtggtc gttctggacg tcaagggtgat
2051 aaaggggata gtcgcttcta ttatcatta caagatgaat taatgatcgc
2101 ttttggttct gaacgtttac agaaaatgat gagccgacta ggtttagatg
2151 actctacacc aattgaatca aaatgggtat caagagctgt tgaatcagca

FIG. 8B

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2201 caaaaacgtg tagaaggtaa taactcgac gcgcgtaaac gtatcttaga
2251 atacgatgaa gtattacgta aacaacgtga aattatctat aacgaaagaa
2301 atagtattat tgatgaagaa gacagctctc aagttgtaga tgcgaatgcta
2351 cgttcaacgt tacaacgtag tatcaattac tatatttaata cagcagatga
2401 cgagcctgaa tatcaacccat tcatcgacta cattaatgac atcttcttac
2451 aagaaggtag cattacagag gatgatataa aaggtaaaga tgetgaagat
2501 attttcgaag tcgtttgggc taagattgaa gcagcatatc aaagtcacaaa
2551 agatatctta gaagaacaaa tgaatgagtt tgagcgtatg attttacttc
2601 gttctattga tagccattgg actgatcata tcgacacaaat ggatcaatta
2651 cgtcaaggta ttcaacttacg ttctttatgca caacacaaate cattacgtga
2701 ctatcaaaat gaaggctcatg aattatttga tatcatgatg caaataatlg
2751 aagaagatoc ttgtaaatlc attttaaat ctgtagtaca agttgaagat
2801 aatattgaac gtgaaaaaac aacagagttt ggtgaagcga agcacgtttc
2851 agctgaagat ggtaaagaaa aagtgaacc gaacccaate gttaaaggcg
2901 atcaagttgg tcgtaacgat gattgtccat gtggtagtgg taaaaaatc
2951 aaaaattgcc atggaaaata aatgatataa aataactcct tccaattaaa
3001 cacctatagt ttgtgttatg ggagggtct ttttatttta caagcgttaa
3051 atactttaaa aatgtgaag aagttgttaa acgttggtat gtacttagtt
3101 ttaaaaaate gtttaggca tatg

FIG. 8C

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1  cttgaacggt acttcaactaa tgtgccgaat gtgaatgcac atgtaaaagt
51  gaaaacttat gcaattcta gcacaaatc gaagtacaa ttccgcttaa
101  tgacgtgaca cttegtgcag aagaagaaa cgatgattta tgctgggaatt
151  gacaagatca ctacaaatt agaattgcaa gttegtaat acaaaacacg
201  tgtcaatcgt aagaacgta aagaagcga acatgaacca ttcccagcaa
251  ctccggaaac tccgcggaa acagctgttg atcatgataa agatgatgaa
301  attgaatatc tccgttctaa acaattcagc ttgaaaccaa tggattctga
351  agaagcggta ttacaaatgg atttacttgg tactgatttc ttcatcttca
401  atgaccgtga aactgatggt acaagcattg ttaccgccc taaagacgga
451  aatatatggt tgattgaac tgtgaaaaa ctaatatgtg atatttgaaa
501  gggctcttgc tgcattttct gctgcaagag ttctttttt tgagaaagcc
551  ctattattaaga tttgattaat aaaaatacaa ttgattgatt tacacggggt
601  glccatgtca aataagagg gatgtattaa gtccataatt gtaatgtgag
651  ctccgatgag tgagcggcat atgattatga talccatgtg gcacatgatg
701  ttaacaaaaa gagaatgaaa ctgtgagaag tacatcttga taaacacaa
751  taggcagttt attaaaaat aatgaacagt atcctatgag tttttaagta
801  taatttaagc catataaalg gtaagataaa ttgttgtaag ccaaacagtt
851  ttatatacca aggagcgaac agaattgggtt ttttaacaaa aattgttgac
901  ggcaataaga gagaatacaa acgectaagt aagcaagctg acaagtaaat
951  ctcaattagaa gaagaatgt caattcttac tgatgaagaa attagaata
1001  aacacaaagc attccaagaa agattgcaag cagaagaaca tgtaagcaaa
1051  caagataaaa ttttagaaga aatat tacct gaagcatttg cgcttggtccg
1101  tgaaggagct aaacgtglat ttoatatgac acctatcca gtccaatatca
1151  tgggtggtat cgccattcat aatggtgaca ttacagaat gagaacaggt

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FIG. 9A

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1201 gaaggtaaaa cattaactgc aacgatgccg acttatttaa acgccttagc
 1251 agcgcgtggt gtgcatgtta ttacagtcac tgactacttg gcaagtcttc
 1301 aaagagaaga aatggccgag ttatatattt tccttggttt atcagtcgga
 1351 ttgaacttga acagcttate acagaaaca aagcgtgaag cttataatgc
 1401 agatatttac tataglacaa ataattgaatt aggettcgac tatttacgcg
 1451 ataacatggt gaattattca gaagaacgtg ttatgcgtcc gcttcatttc
 1501 gctatcattg atgaggtcga ctctatttta atcgatgaag cgcgtacacc
 1551 attgattatt tcaggggaag ctgaaaaac acatctcttt tatacacaag
 1601 caaatgtttt cgtataaatg ttaaaagcag aagatgatta taattatgat
 1651 gaaaaaaca aatcagtlca attaacagat caagggtctg ataaagctga
 1701 acgtatgttc aagttagata acttatatga tttgaaaaac gttgatatta
 1751 tcaegcatat caatcacgca ttacgtgcta actatacatt gcaacgcgat
 1801 gtagattaca tggttgtaga tggagaagta ttgattgtcg accaatttac
 1851 oggtcgaacd atgccaggtc gtcgattctc tgaaggactt caccaaagca
 1901 ttgaggctaa agaaggggtt caatttcaaa atgaatctaa aacaatggct
 1951 tctatcacat tccaaaacta ctccgtatg tataataaat tagccgggtat
 2001 gacaggtaet gctaaaacag aggaagaaga attccgtaac atttataata
 2051 tgacagttac acaatttcca acgaaccgtc ctgttcaacg tgaagataga
 2101 cctgacttga ttttcatcag ccaaaaaggc aagttcgatg ctgttggtga
 2151 agatgttgtt gaaaaacata aaaaaggcca accaatcttt ttaggtaactg
 2201 tagcggttga acaagtgaa tacatttcac aactattgaa aaaaecgggt
 2251 gtgcgtcatg atgtctttaa cgctaaaaac catgaacgcg aagctgaat
 2301 cgtatctaca gcaggtcaca aaggtgcagt cacaatcgca acaaacatgg
 2351 ctggtcgtgg taccgalatt aattaggcg aaggtgttga agaattaggc
 2401 ggccttgctg ttattggtag agaacgtcat gaatcacgcc glatcgatga

FIG. 9B

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2451 tcagttgct ggtcggtctg gacgacaagg tgaccgcgga gaaagccgtt
2501 tctatttctc attacaagat gagtgtatgg tacgtttcgg ttctgaacgt
2551 ctgcaaaaaa tgatgggccg attaggtatg gatgactcta caccgatlga
2601 atcaaaaaatg gtatctcgag ctgttgaatc tgcacaaaaa cgtgttgaag
2651 gtaacaactt cgtgcacgt aaacgtatct tagaatacga tgaagtttta
2701 cgtaaacacac gtgaatcat ttatggtgaa cgtaataata ttatcgattc
2751 agaatacaagt tctgaattag tcattacaat gatcgcctct acattagatc
2801 gtgcaatcag ttattatgta aatgaagoat tggaggaat tgactatgcy
2851 ccgtttatta attttgtgga agatgttttc ttdecaggaag gtgaagtcaa
2901 agaagatgaa atcaaaaggta aaggtaaaga tcgtgaggat attttcgata
2951 cagtatgggc taataattgaa aaagcttatg aagcacaaaa agccaatata
3001 cccgaccaat tcaatgaatt cgaacgtatg attttattac gttctattga
3051 tgggaagatgg acagaccata tcgatacaat ggaatcaatta cgtcaaggta
3101 tccattttacg ttcatatcgt caacaaaaac cacttcgca ctatcaaat
3151 gaagggcacc aactatttga tacaatgatg gtcaatattg aagaagacgt
3201 cagcaaatat atcttgaat caattatcac agtagatgat gatattgaac
3251 gtgataaagc aaagaatat caaggacaac atgtatcagc tgaagatgga
3301 aaagaaaaag taaaaccgca accagttgtt aaagataatc acatcggaag
3351 aatgatcct tgtccatgcy gcagcggtaa aaagtataaa aattgctgcy
3401 gtaaatagta agttgtatta ggaccactgt taatatagctt taagagagat
3451 gctcaattga aattgggtta tctttctaa ggetgtcagc ggctttttt
3501 caatccaaca aaatatgga tatatgctaa aataatagag taatctggaa
3551 aattaaactg gaattggaga gatatgaaa tggaaattat

FIG. 9C

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1  cagteaagt cgetcttctgt gaccgagcca atggacggaa aggtgcccgc ctcccagatc
61  atgaacctcc tagtgtaagc ctataagaag ggccttaaga cggggctcta ctactgcaag
121 atccgcaagg ccaccaacaa cggcgtcttc acgggcggcg accctgtgtg ctctgggtgc
181 caccctgtagc gacgcgcgc gagcgcgatg gccgaggcgg gggacgcggc gacctcacg
241 cgtaaataca aatactttta cgagaccgag tgcctccgacc tagatcaactt gcggtcgtc
301 agcgtcgcaa accgctggct ggagaccgag ttccccctag cggacgacgc caaggacgtg
361 gcgcggctca gcggcgccga gctggagtctt taccgcttcc tgttcgcgtt cctctcgcc
421 gccgatgacc tcgtgaacgt caacctcggt gacctgtccg agctgttcc ccaaaaagac
481 atcctgcatt actatatcga gcaggagtcc atcgaaagtgg tgcactcgcg ggtgtacagc
541 gccatacagc tgetgtctct tagaaacgac gcggtggcgc gcgcgggcta cgtagagggc
601 gccctcggcg acccgcggtt ccggcgcaag gtggactggc tcgagcggcg cgtggccgcg
661 gcagagtcgg tggccgaaaa gtacgtgtc atgattctaa tcgagggcat tttttcttcc
721 tcctcgtttg cggcgattgc ctacctgcgc acccaaaccc ttttcgtcgt gacgtgccaa
781 accaacgacc tcatacgccg cgacgaagcc gtgcacacgg ccgcgtcgtg ctgcattctc
841 gacaaactacc tcggcgggga gcggccgcgcg ccggcccgcga tctacgagct gttccgcgaa
901 gcgtgggaat tgagcgcgag ttatttttgt tgcgcgcgcg gcggcagtcg tatacttgac
961 gtggaggcta ttctcgcgta cgtcaggtac agcgcggacc gcctgctcgc tgcataccag
1021 ctgcctctc tgtttggcac ccgcctcct gggaccgatt ttcctttggc cctgatgact
1081 gccgagaagc acacgaactt ctttgagcgc cgcagcaccg actacacagg caccgtaac
1141 aacgacctgt agggcacccc cgtgccttg ccagagcgcc ccgccttcc tcctcctct
1201 cccccccag ccgcgaataa aaatgttcc atgtcaacga aa

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FIG. 10

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1 tcgagccccg cgaaaccgc cgcgtctgtt gaaatggcca gccgcccagc cgcctctct
61 cecgtcgaag cgcgggcccc ggttggggga caggaggccg gcgccccagc cgcagccacc
121 cagggggagg ccgcggggc cctctcgcc cccggccacc acgtgtactg ccagcgagtc
181 aatggcgtag tggcgcttc cgacaagacg cccgggtccg cgtcctaccg cctcagcgat
241 agcaactttg tccaatgtg ttccaactgc accatgatac tcgacggaga cgtggtgcgc
301 gggcgcccc aggacccggg ggcgcggca tccccgcctc cctcggtgc ggtgacaaac
361 atcggagccg gcagcgacgg cgggaccgcc gtcgtggcat tcgggggaac cccacgtcgc
421 tcggcgggga cgtctaccgg taccagacg gccgacgtcc ccaccgaggc ccttgggggc
481 cccctctc ctccecgctt caccctgggt ggcggctgtt gttcctgtcg cgacacacgg
541 cgccgctctg cggtatctcg gggggagggg gatccagtcg gccccgcgga gttcgtctcg
601 gacgaccggt cgtccgattc cgaetcggt gactcggagg acacggactc ggagacgctg
661 tcacacgct cctcggacgt gtcggcggg gccacgtacg acgacgcct tgaetccgat
721 tcgtcatcgg atgaatccct gcagatagat ggcgccgtgt gtcgcccgtg gagcaatgac
781 accgcgcccc tggatgtttg cccgggacc cccggcccgg gcgcccagc cgttggtccc
841 tcagcggtag acccacacgc gccgacgcca gaggccggcg ctggtcttgc ggcgatccc
901 gccgtggccc ggaagagacgc ggaagggttt tcggaccccc gccacgtctt gggacggggc
961 acggcctacc cgtccccct ggaactcacg ccgagagaacg cggaggccgtt ggcgcgcttt
1021 ctgggagatg ccgtgaaccg cgaaccccg ctcattgttg agtaactttg ccggtgcgccc
1081 cgcgaggaaa ccaagcgtgt ccccccagg acattcgga cccccctcg cctcacggag
1141 gacgactttg ggtcttcaa ctacgcgtc glggagatgc agcgcctgtg tctggacgtt
1201 cctccggtcc cgccgaacgc atacatgccc tattatctca gggagtatgt gacgcggctg
1261 gtcaacgggt tcaagccgt ggtgagccgg tccgtcgcc ttaccgcct cctgggggtt
1321 ctggtgcacc tgcggatccg gaccggggg gcctcctttg aggagtggct gcgatccaaag

FIG. 11A

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1381 gaagtggccc tggattttgg cctgaeggaa aggttcgcg agcaggaagc ccagctggctg
 1441 atcctggccc aggtcttggg ccattacgac tgtctgaccc acagcacacc gcacacgctg
 1501 gtcgagcggg ggtgcgaatc ggcctgaag tatgaggagt ttacctaaa gcgtttggc
 1561 gggcaactaca tggagtccgt cttccagatg tacacccgca tcgccggctt ttggccctgc
 1621 cgggccaccg cgggcatgcg ccacatcgcc ctggggcgag aggggtcgtg gtgggaaatg
 1681 ttcaagttct ttttccaccg cctctacgac caccagatcg taccgtcgac ccccgccatg
 1741 ctgaacctgg ggaccccgaa ctactacacc tccagctgct acctggtaaa cccccaggcc
 1801 accacaaca aggcgacct gcgggccatc accagcaacg tcagtgccat cctcgccccg
 1861 aacgggggga tcgggctatg cgtgcaggcg tttaacgact ccggccccgg gaccgccagc
 1921 gtcatgcccc cctcaagggt ccttgactcg ctggtggcg cgacacaaca agagagcgcg
 1981 cgtccgaccg gcgcgtgcgt gtacctggag ccgtggcaca ccgacgtgcg ggcgtgctc
 2041 cggatgaagg gggtcctcgc cggcgaagag gccacgcgt gcgacaatat cttcagcgcc
 2101 ctctggatgc cagacctgtt ttcaagcgc ctgattcgcc acctggacgg cgagaagaaac
 2161 gtacacatgga cctgtttcga ccgggacacc agcatgtcgc tcgccgactt tcacgggggag
 2221 gagtctgaga agctctacca gcacctcgag gtcatggggt tcggcgagca gataccatc
 2281 caggagctgg cctatggcat tgtgcgcagt gcggccacga ccgggagccc ctctgtcatg
 2341 ttcaaaagacg cggtgaaaccg ccactacatc tacgacaccc agggggcgccg catcgccggc
 2401 tccaacctct gcaccgagat cgtccatccg gcctccaagc gatccagtgg ggtctgcaac
 2461 ctgggaagcg tgaatctggc ccgatgcgtc tccaggcaga cgtttgactt tggcgggctc
 2521 cgcgacgccc tgcaggcgtg cgtgctgatg gtgaacatca tgatcgacag caccgtacaa
 2581 cccacgcccc agtgaccccg cggcaacgac aacctgcggt ccatgggaat cggcatgcag
 2641 ggcctgcaca cggcctgcct gaagctgggg ctggatctgg agtctgccg atttcaggac
 2701 ctgaacaaac acatcgccga ggtgatgctg ctgtcggcga tgaagaccag caacgcgctg

FIG. 11B

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2761 tgcgttcgcg gggcccgctcc cttaaccac tttaagcgca gcatgtatcg cgcgggcgcg
2821 tttaactggg agectttcc ggacgcccgg ccgcggtacg agggcgagtg ggagatgcta
2881 cgccagagca tgatgaaca cgccctgcgc aacagccagt ttgtcgcgt gatgcccacc
2941 gccgcctcgg cgcagatctc ggacgtcagc gagggtttg cccccctgtt caccaccctg
3001 ttcagcaagg tgacccggga cggcgagacg ctgcgcccc aacgctcct gctaaaggaa
3061 ctggaacgca cgtttagcgg gaagcgctc ctggaggtag tggacagtct cgacgccaag
3121 cagtgttcgg tgccgcaggc gctcccgctc ctggagccca cccacccct ccggcgattc
3181 aagaccgcgt ttgactacga ccagaagtgg ctgategacc tgtgtgcgga ccgcgcccc
3241 tacgtcgacc atagccaac catgacctg tatgtcacgg agaaggcgga cgggacccctc
3301 ccagccctcc ccctgggtccg ccttctgtc cagcatata agcgcggact aaaaacaggg
3361 atgtactact gcaagggtcg caaggcgacc aacagcgggg tctttggcgg cgacgacaac
3421 attgtctgca tgagctgcgc gctgtgaccg acaaaccccc tccgcgccag gccgcgcgc
3481 actgtcgtcg ccgtcccaag ctctccctg ctgccatg

FIG. 11C

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1  gtgtgtttgg  cgtgtgtctc  tgaatggcg  gaaccacac  tgcaatggg  attcatggac
61  acgttacacc  cccctgactc  aggatagg  catatctctc  ttgattgac  tcagcacacg
121  atcgaccccc  accctgtgt  gccgggata  aagccaacg  cgcggtct  gggttaccac
181  aacaggtggg  tgettcgggg  acttgacgt  cgcaactctc  ctgcgagccc  tcacgtcttc
241  gccacccgat  tctgtttcgg  ttctgtcgg  ccggtgtgt  cctgtcgaca  gattgttggc
301  gactgccccg  gtgattcgtc  ggccggtgcg  tcttttcgg  cgtaccgccc  accccgcctc
361  caacggggcc  gccgtgttt  ccgttcacg  cgtccgagcc  accgtcaact  tggttccaat
421  ggccaaccgc  cctgccgcat  ccgccctgc  cggagcgcg  tctccgtccg  aacgacagga
481  accccgggag  cccgaggtcg  cccccctgg  cggcgaccac  gtgttttga  ggaagtcag
541  cggcgtgatg  gtgctttcca  gcgatcccc  cgcccccg  gcctaccgca  ttgcgcacg
601  cagctttgtt  caatgcggt  ccaactgcg  tatgataatc  gacggagacg  tggcgcgcgg
661  tcatltgcgt  gacctcgagg  gcgtacgtc  caccggcgcc  ttctgcgca  tctcaaacgt
721  cgcagccggc  ggggatggcc  gaaccgct  cgtggcgctc  ggcggaacct  cgggcccgtc
781  cgcgactaca  tccgtgggga  ccagacgtc  cggggagttc  ctccacggga  acccaaggac
841  ccccgaaacc  caaggacccc  agctgtccc  cccgccccct  cctccccct  ttccatgggg
901  caacgagtgc  tgcgccgtc  gcgatgccg  gggcgcgcc  gagaaggacg  tcggggccgc
961  ggagtcattg  tcagacggcc  cgtcgtccg  ctccgaaacg  gaggactcgg  actcctcggg
1021  cgaggatacg  ggtcgggtt  cggagacgt  gtctcgatcc  tcttcgatct  gggccgcagg
1081  ggcgactgac  gacgatgaca  gcgactccg  ctgcggtcg  gacgactccg  tgcagccccg
1141  cgttgtcgtt  cgtcgagat  ggagcgacgg  ccctgcccc  gtggccttct  ccaagccccg
1201  gcgccccggc  gactccccg  gaacccccg  cctgggcgcc  ggcacccggc  cgggtccgc
1261  gacggacccg  cgcgcgtcgg  ccgactccg  ttcgcggcc  caccgcggc  cccccaggc
1321  ggacgtggcg  ccggttcttg  acagccagcc  cactgtggga  acggaccccc  gctaccacgt

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FIG. 12A

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1381 cccctagaa ctacgcccg agaacgcgga ggcggtggcg cggtttctgg gggacgccgt
 1441 cgaccgcgag cccgcgctca tcttgagta cttctgtcgg tgcgcccgcg aggagagcaa
 1501 gcgcgtgccc ccacgaacct tcggcagcgc ccccgcctc acggaggacg actttgggct
 1561 cctgaactac gcgctcgtg agatgcgacg cctgtgcctg gacctcccc cggtcecccc
 1621 caacgcatac acgcccatac atctgagga gtatgcgacg cggctgggta acgggttcaa
 1681 acccctggcg cggcggtccg cccgcctgta tcgcatcctg gggattctgg tcaacctgcg
 1741 catccgtacc cgggaggcct cctttgagga atggatgcgc tccaaggagg tggacctgga
 1801 cttcgggctg acggaaggc ttgcggaaca cgaggcccag ctaatgatcc tggcccaggc
 1861 cctgaacccc tacgactgtc tgatccacag caccgccgaac acgctcgtcg agcgggggct
 1921 gcagtcggcg ctgaagtacg aagagtttta cctcaagcgc ttccggcggc actacatgga
 1981 gtccgtcttc cagatgtaca cccgcacgcg cgggttcctg gcgtgccggg cgacccgcgg
 2041 catgcgccac atcgcctgg ggcgacaggg gtctgtgtgg gaaatgttca agttctttt
 2101 caaccgcctc tacgaccacc agatcgtgcc gtccaccccc gccatgtga acctcggaac
 2161 ccgcaactac tacacgtcca gctgatacct ggtaaacccc caggccacca ctaaccaggc
 2221 caccctccgg gccatcaccg gcaacgtgag cgccatcctc gccgcgaacg ggggcacatcgg
 2281 gctgtgcatg caggcgttca acgacgccag ccccggcacc gccagcatca tgcgggccct
 2341 gaaggtcctg gactccctgg tggcggcgca caacaaacag agcacgcgc ccaccggggc
 2401 gtgcgtgtac ctggaacctt ggcaacgcga cgttcgggccc gtgtcagaa tgaaggcggt
 2461 cctgcgccgc gaggaggccc agcgtgcga caacatcttc agcgcctctt ggatgccgga
 2521 cctgttcttc aagcgctga tccgccacct cgacggcgag aaaaacgtca cctgggtccct
 2581 gttcgaccgg gacaccagca tgtcgtcgc cgactttcac ggcgaggagt tcgagaagct
 2641 gtacgagcac ctcgaggcca tggggttcgg cgaaacgac cccatccagg acctggcgta
 2701 cgccatcgtg cgcagcgcg ccaaccacgg aagcccctc atcatgttta aggaecgggt

FIG. 12B

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2761 aacagccac tacatctacg aacgcaagg ggcggccatt gccggtcca acctctgcac
 2821 ggagatcgtc caccgtcct ccaacgctc cagcgggtc tgcaacctgg gcagcgtgaa
 2881 tctggcccgat tgcgtctccc ggcggaagt ttcgatttggc atgctccgag acgcccgtgca
 2941 ggcgtgcgtg ctaatggtta atatcatgat agacagcag ctgcagccga cgcgccagtg
 3001 cgcccgccgc cagcaaac tgcggtccat ggcattggc atgcagggcc tgcacacggc
 3061 gtgcctgaag atggcctgg atctggagtc ggcgagttc cgggacctga acacacacat
 3121 cgccgaggtg atgctgctcg cgcccatgaa gaccagtaac gcgctgtgcg ttgcgcgggc
 3181 gcgtcccttc agccacttta agcgcagcat gtaccgggc gtaccgtttc actgggagcg
 3241 ctlttctgaac gccagcccgc ggtacgaggg cgagtgggag atgctacgcc agagcatgat
 3301 gaaacacggc ctgcgaaca gccagttcat cgcgtcatg ccacccggcg cctcggccca
 3361 gatctcggac gtcagccagg gctttgccc cctgttccc aacctgtca gcaagggtgac
 3421 cagggaacgc gagacgtgc gcccacaac gctcttctg aaggaaactcg agcgcacgtt
 3481 cgccgggaag cggtctcttg acgcgatgga cgggtctgag gccaaagcagt ggtctgtggc
 3541 ccaggccctg ccttgccctg accccgccc tgcagaccgc gccccctatg ttgatcacag
 3601 ctacgaccag gaactgtga tgcacctgtg ggcggaacggg acgctccccg cctccaccct
 3661 ccaatccatg actctgtatg tcaacagaa ggcggaacggg acggtggatgt actactgcaa
 3721 ggtccgcctt ctgctccag cgtaccaca gcggggtgtt cgccggcgac gacaaacatcg tctgcacaag
 3781 ggttcgcaag ggcaccaaca gcggggtgtt cgccggcgac gacaaacatcg tctgcacaag
 3841 ctgcgcgtg taagcaacag cgtccgac tcccccgga gacccgaccc cctagatacc cagcgctcgg
 3901 tcgccatgga tccccgcgtc tcccccgga gacccgaccc cctagatacc cagcgctcgg
 3961 ggcccggggc ggcccgatt cggtgtgccc ccccccgga gcggtacttc tacacctccc
 4021 agtgcgccga catcaaccac ctctcgtccc tcagcatcct gaaccgctgg ctggagaccg
 4081 agctcgtgtt cgtcggggac gaggaggac tctccaagct ctccgaggcg gagctcggct

FIG. 12C

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4141 tctaccgctt tctgtttgcc ttctgtcgg ccgcggaaga cctggtgacg gaaaacctgg
4201 gcggcctctc cgccctcttc gaacagaagg acattcttca ctactacgtg ggcagggaat
4261 gcatcgaggt cgtccactcc cgcgtctaca acatcatcca gctggtgctc ttccacaaca
4321 acgaccagge gcgcgcgccc tatgtgccc gaaccatcaa ccaccggcc attcgcgtca
4381 aggtggactg gctggaggcg cgggtgcggg aatgcgactc gatcccggag aagttcatcc
4441 tcatgatect catcgagggc gtcttttttg ccgcctcggt cgcgcgcac cgtacctgc
4501 gcaccaacaa cctcctgagg gtcacctgcc agtcgaacga cctcatcagc cgccacgagg
4561 ccgtgcatac gacagcctcg tctacatct acacaacta cctcgggggc cgcgccaagc
4621 ccgaggcggc gcgcgtgtac cggctgttcc gggaggcggt ggatatcgag atcgggttca
4681 tccgatacca gccccgacg gacagctcta tcttgagtc cggggccctg gcggccatcg
4741 agaactacgt gcgattcagc gcggatcgcc tgctggcct gatccatatg cagccccgt
4801 attcgcgcc cgccccgac gccagcttcc cctcagcct catgtccacc gacaaacaca
4861 ccaactctt cgagtgcgc agcacctcgt acgcggggc cgtcgtcaac gatctgtgag
4921 ggtctgggcg ccttgtagc gatgtctaac cgaataaag ggtcgaac ggactgttgg
4981 gtctccggtg tgattattac gcaggggagg ggggtggcgg ctggggaaag ggaagggaacg
5041 ccggaacca gagaaaagga ccaaaagga acgcgtcca accgataat caagcgccga
5101 ccagaacccc gagatgcata ataacaacg attttattac tcttattatt aacaggtcgg
5161 gcatcgggag gggatggggg cgcgcgttcc ctccgttccg gctactcgtc ccagaattta
5221 gccaggacgt ccttgtaaaa cgcgggcggg ggcgcgtggg ccacaccccg cgccagaaac
5281 cggtcggcga tgtccggggc ggtgatatga cgagtcacga tggagcgccg taaatcttcg
5341 tcgcggaggt cctgatagat gggcagtcct tttagaaggg tccagggtcc ccgctccttg
5401 gggctgataa gcgatatgac gtaattgacg tatctgtgct ccaccagctc ggcgatggtc
5461 atcggatcgg gcagccagtc cagggcctcc ggggcgtcgt ggatgacgtg gcggcgacgt

FIG. 12D

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5521 ccggcgacat agccgcggtg ttccgcgacc cgtgcgcgt tggggacctg caccgctcg
5581 ggcggggtga gtatctccga ggaggacgac cgggcgccgt cgcgcggccc accggcgacg
5641 tccgggggct ggaggggggg gtcttcttcg tagtcgtcct cgcccgcgat ctgttggggc
5701 agaatttcgg tccacgagat gcgcgtctcg agcccgaccg ggccgcgcgt cagcgtaggc
5761 atgctctcca gggagcgaga gttggcgcg tcccgcggg ccgcccgcg gccctgggat
5821 cggctcgggg cggtcacgtg acactcgcgc agcacgtcct cgacgggacgc gtagggtgta
5881 ttgggggtgca ggtctgtgtg gcagcggacg aacagcgcca ggaactgcgg gtaactcacc
5941 ttgaagtacc ctgcag

FIG. 12E

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1 aaaccactgt tctttacact ttatgctcta gtttttggtta atagtgcttt ggaacacttt
 61 taccctaacc gaatttatgg ctttggattt tttagaccac gactgtccac tggggattgt
 121 ttcegatatt atatccaacg tgaataccat caaagagtat ggatattcca gcgaattatc
 181 aacaacgctg gaacctegcc cgtctcgaga acagggtgta gagtatatca ccagagtcgt
 241 ggataaacac aagccgctgt gcagagtcga cgaacgectt tacattgcgt gcggggagct
 301 tgtacacctc cgaattaaag cagcaaacac agacctgaaa tattggetaa aatcgctcga
 361 gattgatctt agcgatgctg tggaaacaggc catattggaa cacattgact ttgttcagaa
 421 aacctcaac tcgtttgaaa catcggaata ccgagatttg tgttcattag gcctgcaatc
 481 tgcgctaagg tatgaagaaa tgtatttagc caaatgcga ggcggacgtc tagagtccat
 541 ggggcaattt ttctctagac ttgcaactac tgcctacgca tatactatgg aacaaccacg
 601 aatggctcgc gtgttggtta gcggtgaggt tggctggaca tatattttca gagccttttt
 661 tactgcgcta gccggacagg ttgtcattcc ggccacgcca attatgctgt ttggtgggag
 721 agactgtggg tctatggcca gctgttattt gctaaacccc agggtaacag atatgaactc
 781 tgcaattccg gctcttatgg aagaggttgg acccattttg tgcaaccgag gaggaaattgg
 841 actgtcttta cagaggttta acactccacc cacagaaggt tgttcacggg gtgtcatggc
 901 tctcctaagg ctactagact ctatgacctt ggccattaac agcgacggtg aaagaccacac
 961 aggagtgtgt gtttatttcg aacctggca cgcagacatc cgcgccattt taaatatgcg
 1021 cggaaatgetg gccagagacg aaactgtgcg ctgcgacacac atctttgctt gtatgtggac
 1081 cccagaccctg ttttttgacc gctatcaacg gtacgtcgat ggagaagcgc gcataatgtg
 1141 gactctgttt gatgatactg categcacct ctgccatatg tacggaaatg atttcacacg
 1201 ggaatatgag cgcctggagc ggtgtggatt tgggatagac gctattccca tacaggacat
 1261 ggcctttatc atagttagaa gtgctgtaat gacagggaagc ccatttttga tgtttaaaga
 1321 cgcgtgcac accgactacc acttgacat gcggcagaga ggtgcgataa tggggctctaa

FIG. 13A

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1381 tctatgcaca gaaattatcc agcatgccga cgaaccacca aacgggggtgt gtaatctagc
 1441 cagcatcaac ctcccdaaat gtctagccct tccacctcca aatatlgcag gtgtgccata
 1501 ttttgacttc gccgctctgg gccgcgtgc cgcactgcc acaatttttg tcaatgcgat
 1561 gatgtgtgcc agcacatata caactgttaa atcccagaa ggcttgaag aaaaccggtc
 1621 gctgggactt ggaattcagg ggtacatac cagtttttg atgctggacc tggatatggc
 1681 atctccagag gcgcaccacc taacaagca atagcagaa aggcgtgtat tgaactctat
 1741 gaaggccagc gcaacgctct gcaagctggg tatgcaacc ttttaagggt ttgaagacag
 1801 caagtacagt cgggggggaa taccctttga tgcctacca aatgtaaac taacaaccg
 1861 caacgcctgg cgtagacttc gcactgacat aaaaacatac ggcttgtaca attctcagtt
 1921 tgtagcctat atgccaacag tatctctgc acaggttacc gagagcagcg aggggttttc
 1981 tcctgtttac acaaacctgt tttagcaagt tactgtacc ggggaagtac tcaggcccaa
 2041 tgtactgcta atgcgcacca tcagaagtat ttttccacag gaatgcgcgc gcttacaagc
 2101 gctatctacg ctagaagctg cgaatggtc agttgtggga gcgtttgggtg atttgccagt
 2161 tggtcacccc ctacagtaagt ttaaacacgc atttgagtac gaccagacta tgctaattaa
 2221 catgtgtgct gacagggtg cgtttgtgga ccagagccaa tccatgtctt tgtttataac
 2281 tgagcctgct gacggaaaac tcccgcctc cagaattatg aatcttttgg tccacgcata
 2341 taacgcgga cttaaaacag gcatgtacta ctgcaaatc aagaaggcaa caaacaacgg
 2401 agtcttttgtt ggcggagacc tagtctgcac cagctgcagc ttgtagggca gcctcgccat
 2461 ttgtgccagg gcgggaaaat aattatggcc ctgaaaaact ctaaaaaaac agattttgct
 2521 gacgagttat tgataaatgc gtatttctat acgccggaat gtcccgatat tgaacaccta
 2581 cgcttgttga gcgttgccaa ccgctggctg gatacggacc ttccaatttc tgaatgacctc
 2641 aaggacgttg ctaaactcgc gccagccgag cgagagtttt accggttttt gtttgccttt

FIG. 13B

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2701 ttatctgctg ctgacgactt ggtaaattta aacctgggag atttatcegc actatttact
2761 caaaggaca ttcttcacta ctacattgag caagagtccta ttgaagtaac gcactccaga
2821 gtatatagcg ctatacagct tatgttgttt ggaacgacg caacagcgcg cgctagggtat
2881 gtcgcactcg ttgtcaaga cgtggccata gacctaaagg tatcttggtt gcaagcaaaag
2941 gtgcgagaaat gcaaatctgt ggcggaaaag tatattttga tgaatatat agaggcgctt
3001 ttctttcgct cgtcccttcc gtccatcgca tatcttgcga cccacaatct ctttgtggtat
3061 acctgtcaaa gtaatgatit aattagccgc gacgaagcaa ttcaacccaa cgcctcgtgc
3121 tgtatctaca acaactacct tgggcgtttt gaaagccag ctccaacgag gatttatgcg
3181 ctgttttctg aggccgtaaa catcgagtgt gaatttttgc ttcccatgc ccccaaaagc
3241 agccacctgt tggacattga agccatcata tgcctacgtac gctatagcgc ggacaggctt
3301 ttgggggaaa ttggactatc tccgctgttt aatgctccca aacccccacc aagcttcccc
3361 ctactgttca tgactgtgga aaacacatacc aacttttttg aaaggcgaaag caccgcatac
3421 tcggggaactc ttataaacga tctgtaatgt aaaaataaaa actaattttg attcaacttat
3481 ttgtcttgtt tgcgtgttgg atgtacgca tttaaaaaaa tactgagaaa agatactccc
3541 gatttaactt tatttaagac cattgtcttc ggtgtccaca gtcactccag tagttaacca
3601 acacagtggt gtaatcagtg ggggtgggaa tgtggttcca aaacatatat gcaagctctc
3661 tgacaatttc gtgttcgg

FIG. 13C

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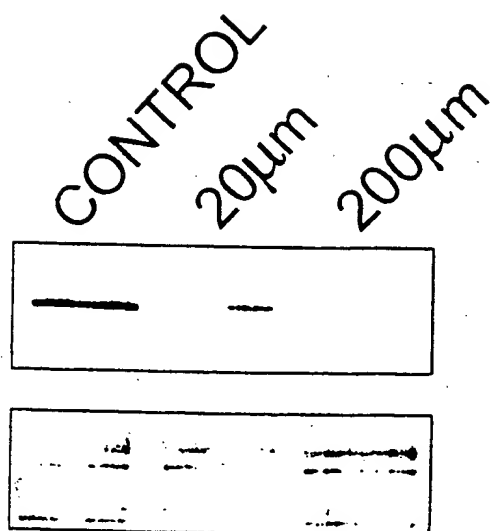
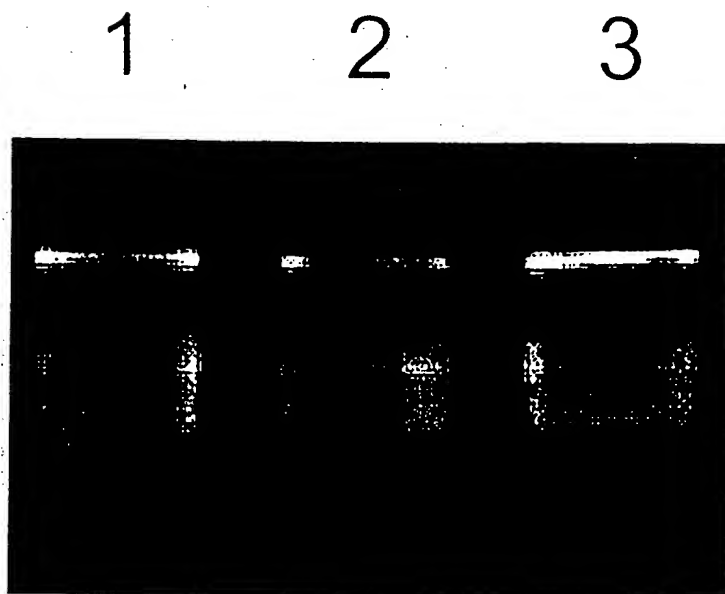


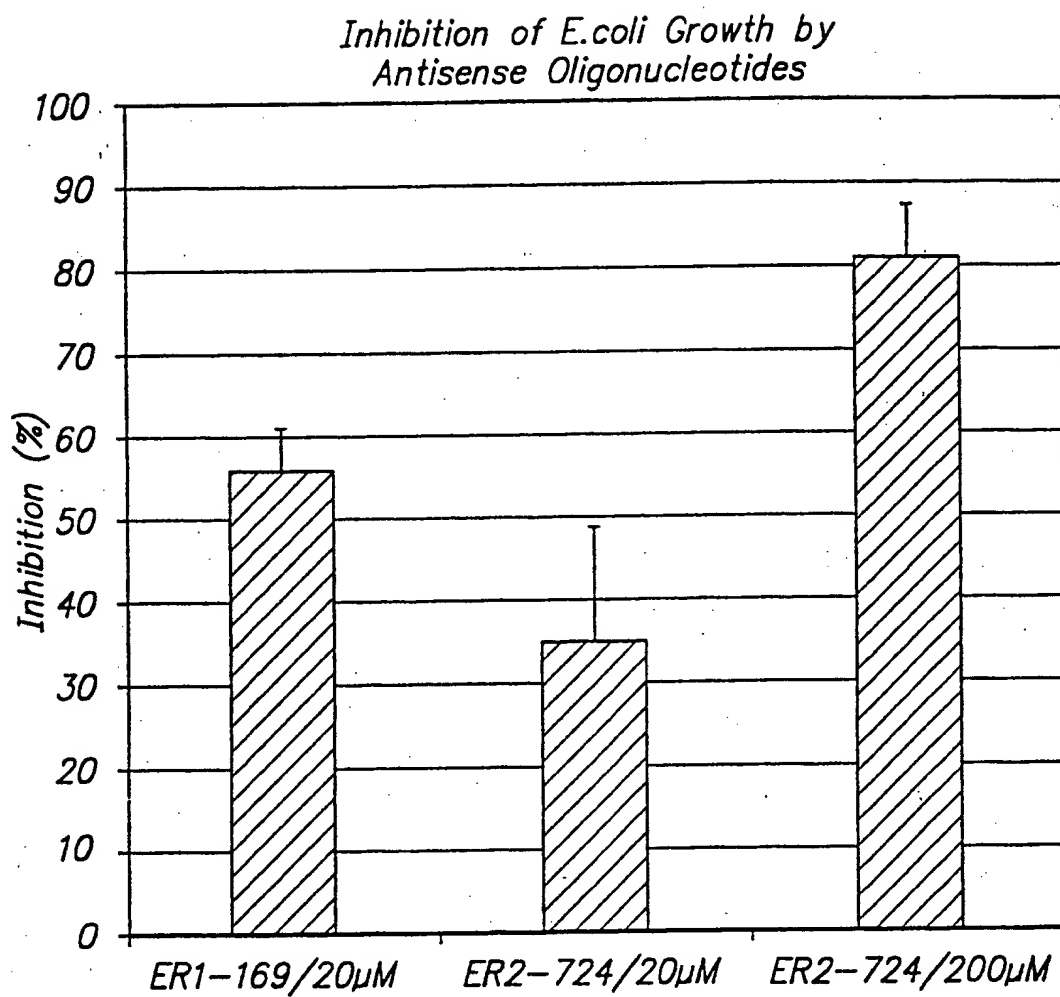
FIG. 14



SecA

FIG. 17

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**FIG. 15**

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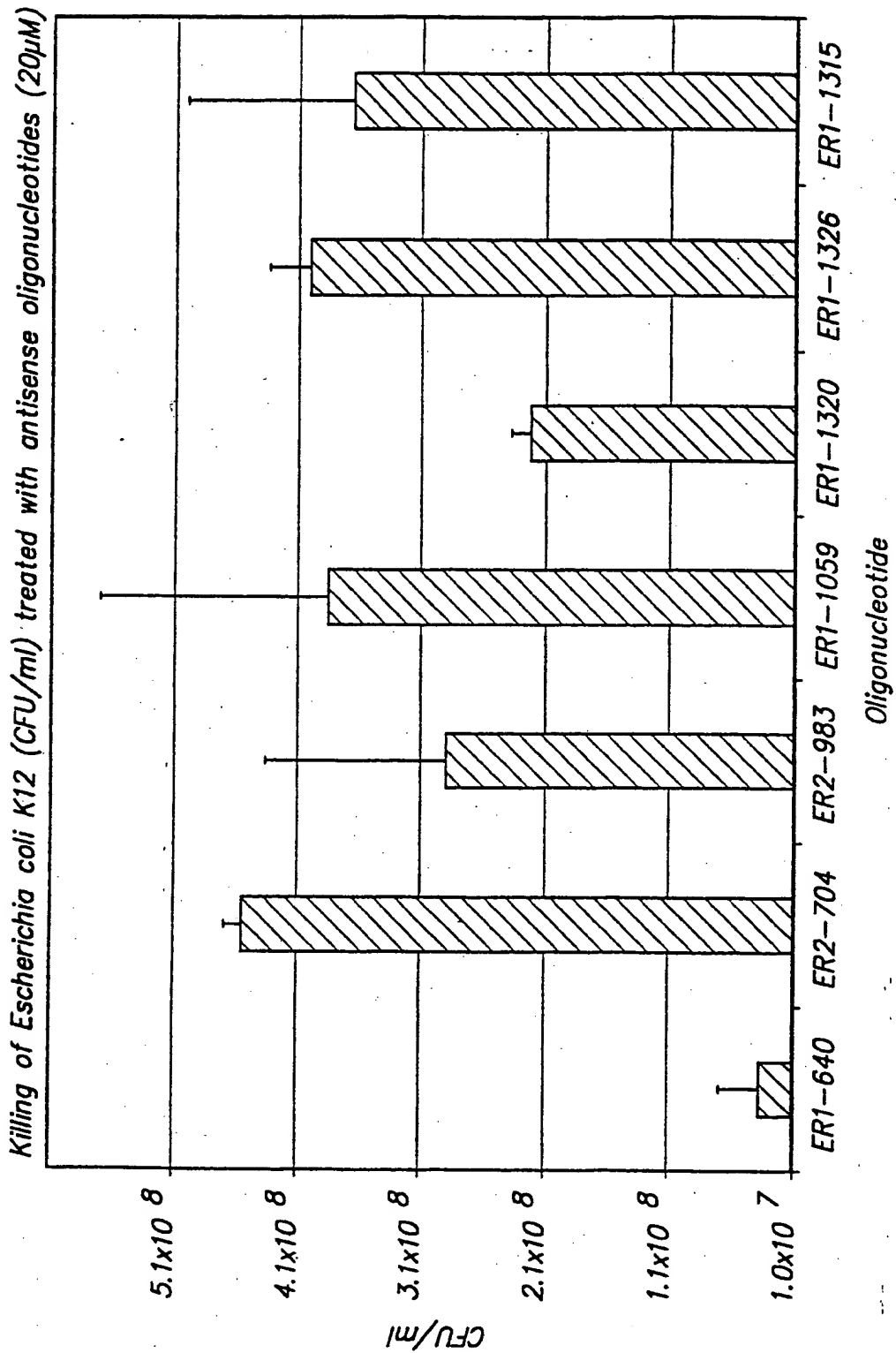


FIG. 16

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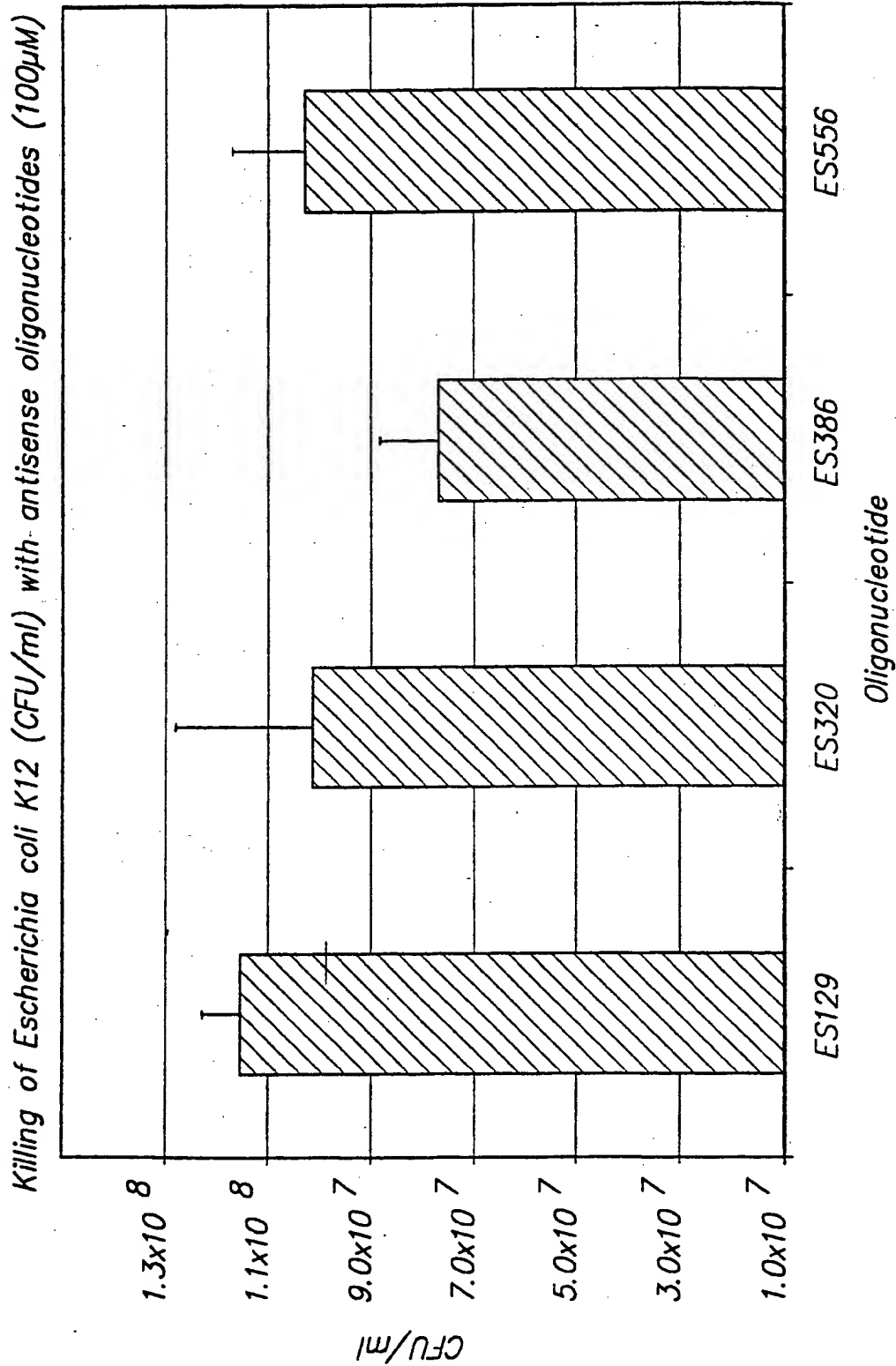
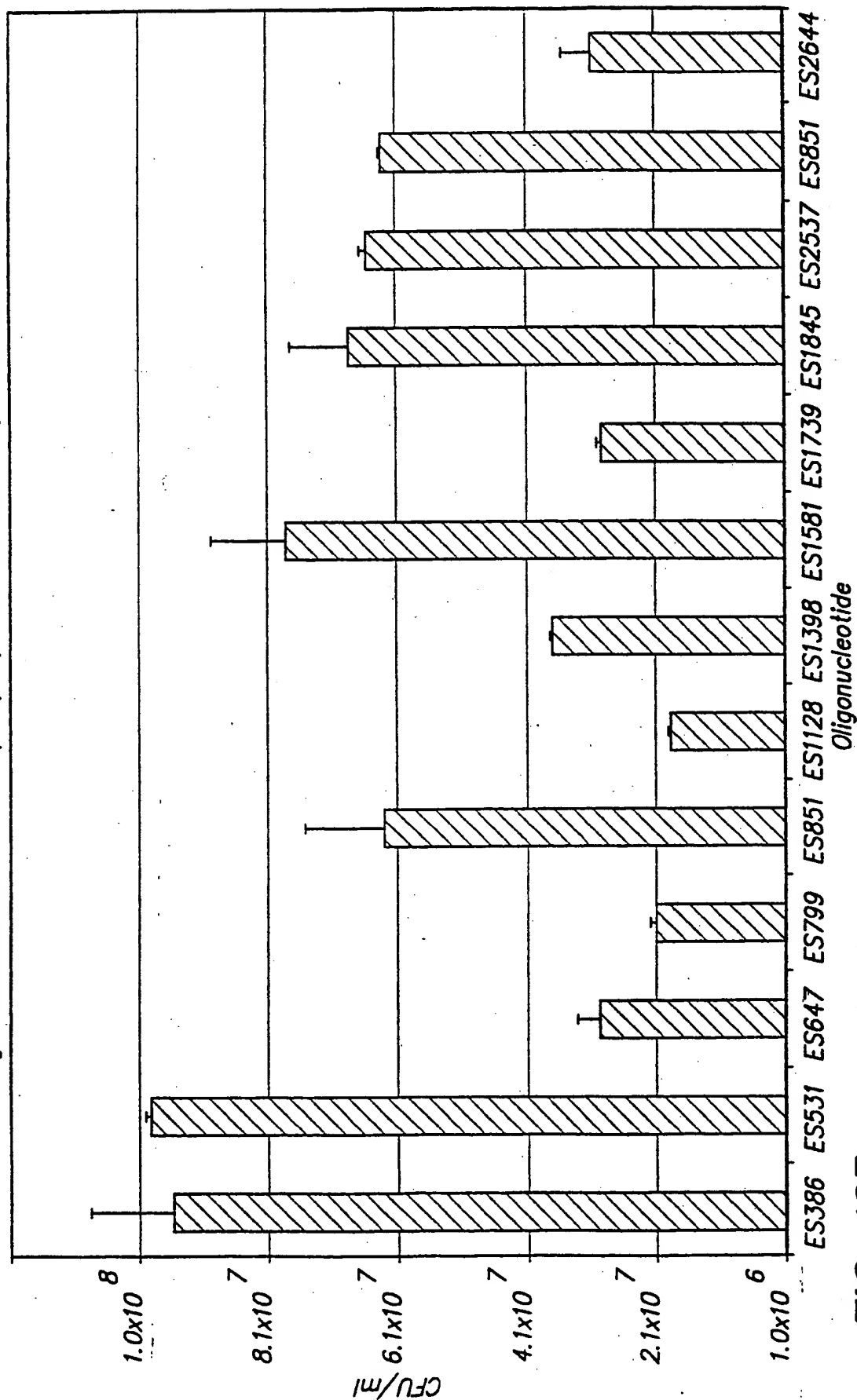


FIG. 18A

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Killing of Escherichia coli K12 (CFU/ml) with antisense oligonucleotides (20µM)

**FIG. 18B**

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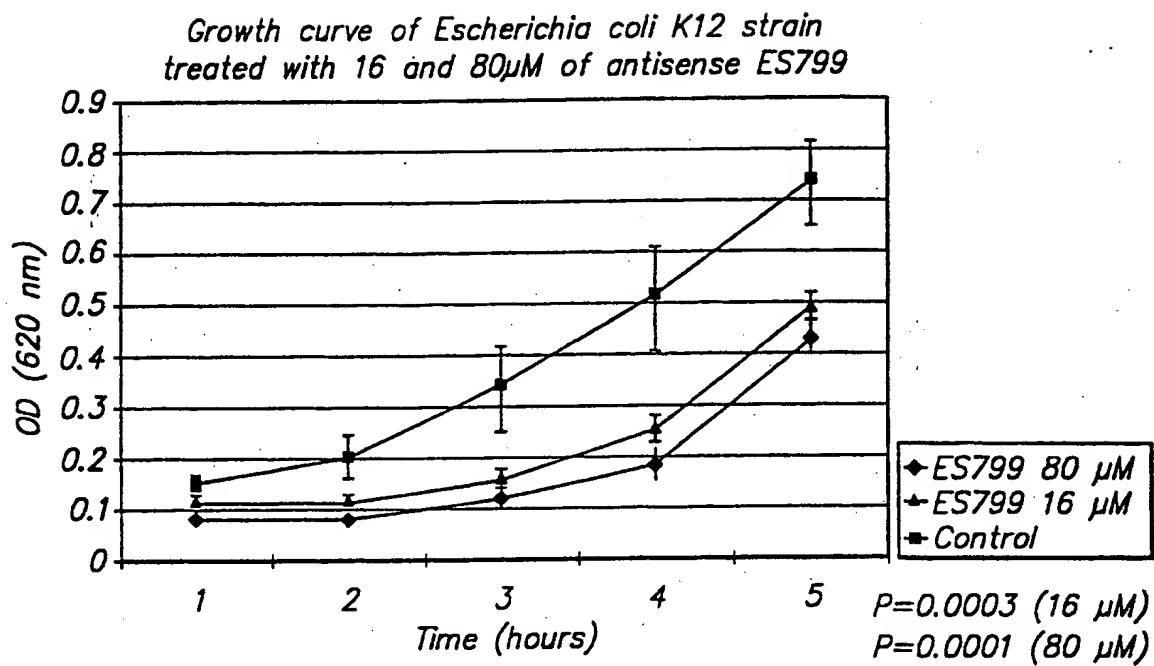
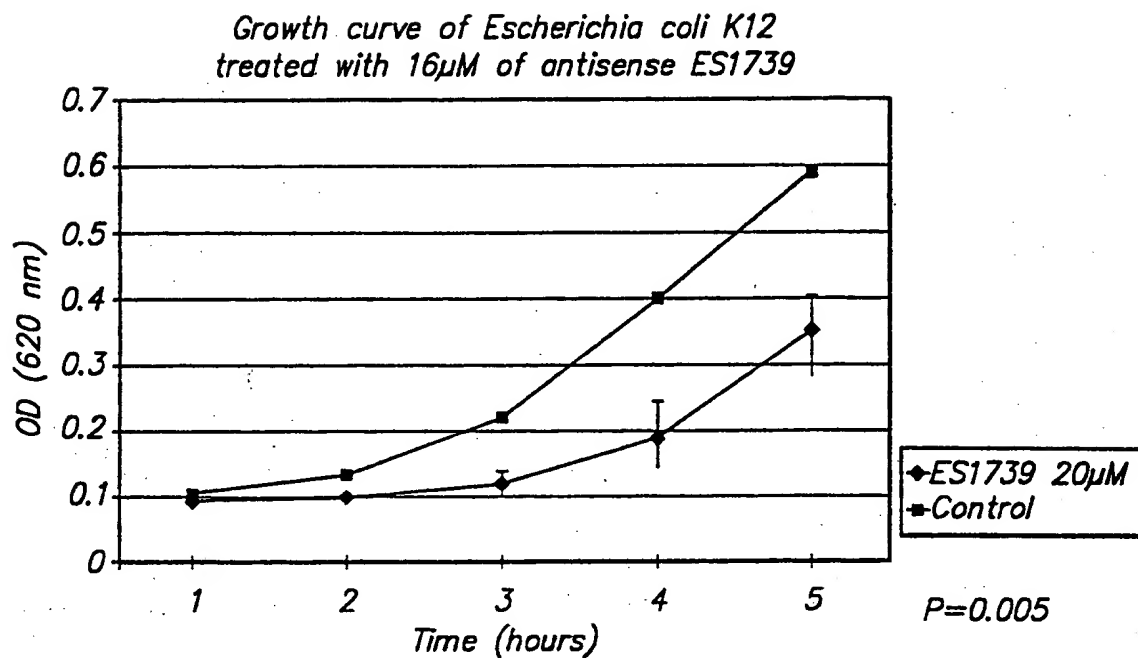


FIG. 19A

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**FIG. 19B**

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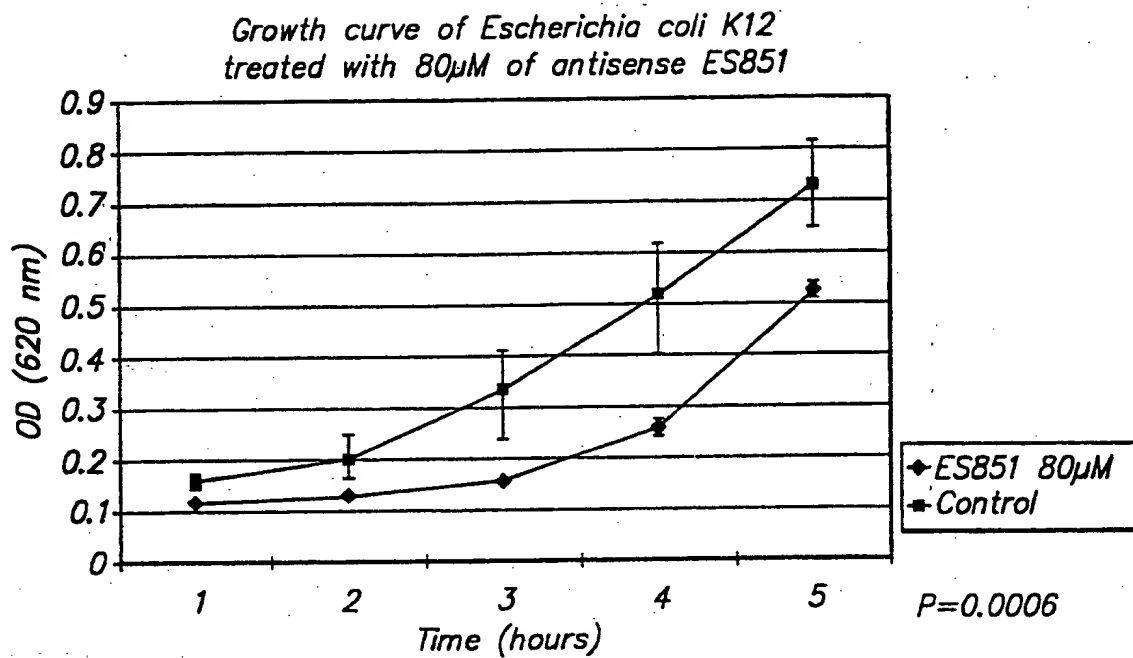


FIG. 19C

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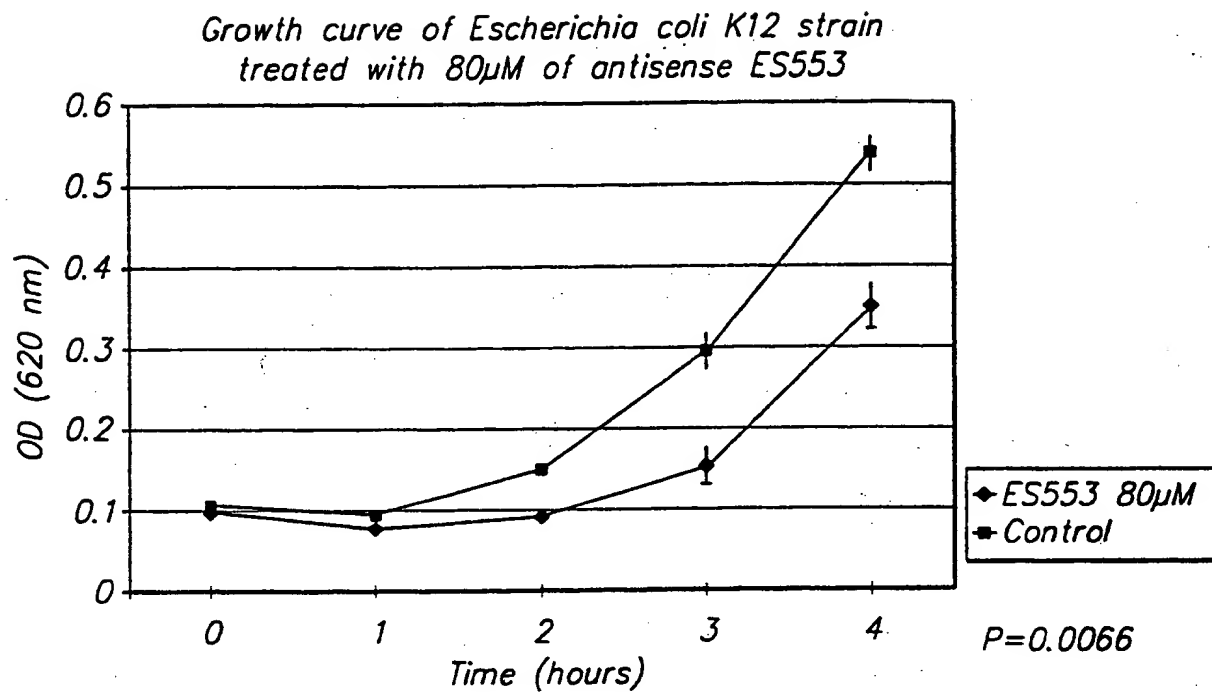
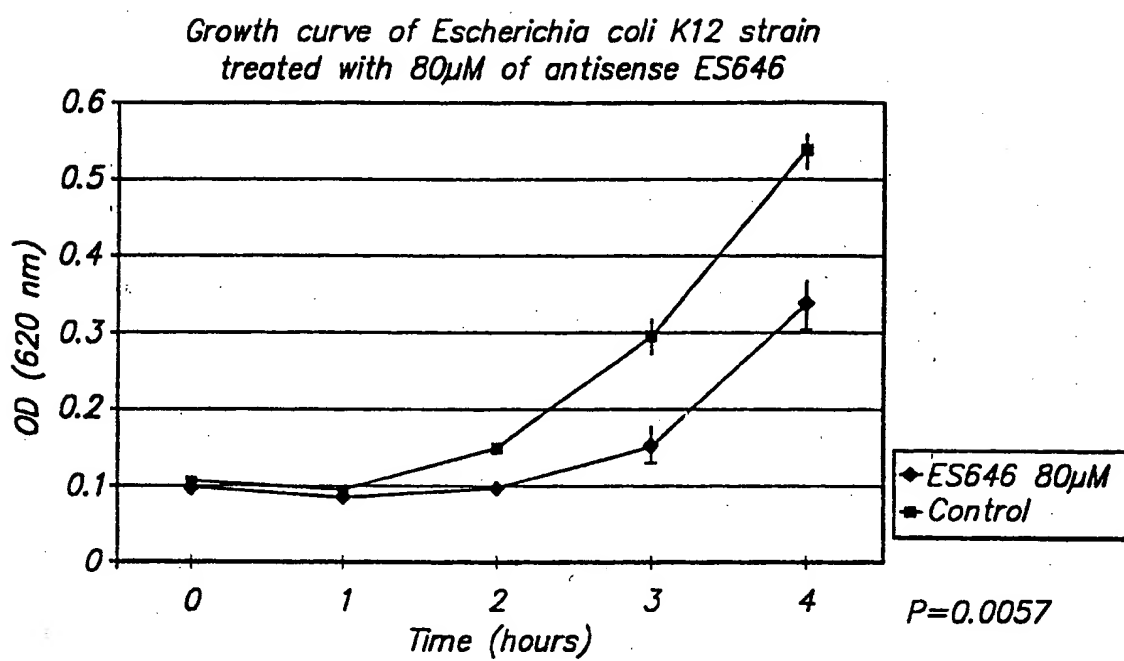
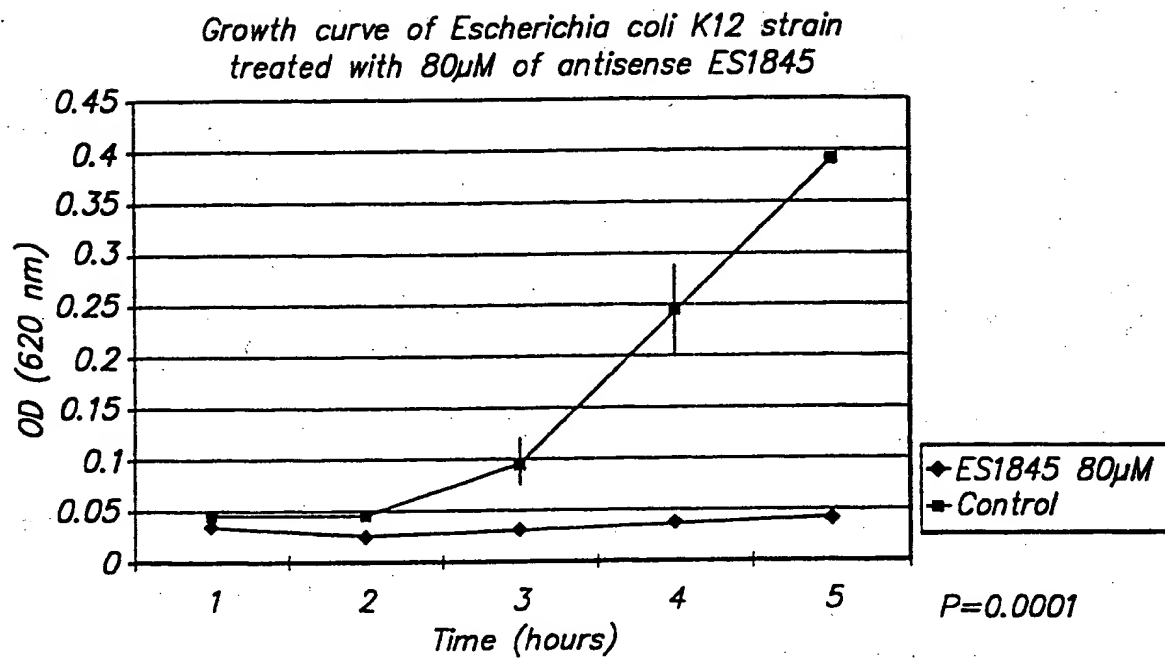


FIG. 19D

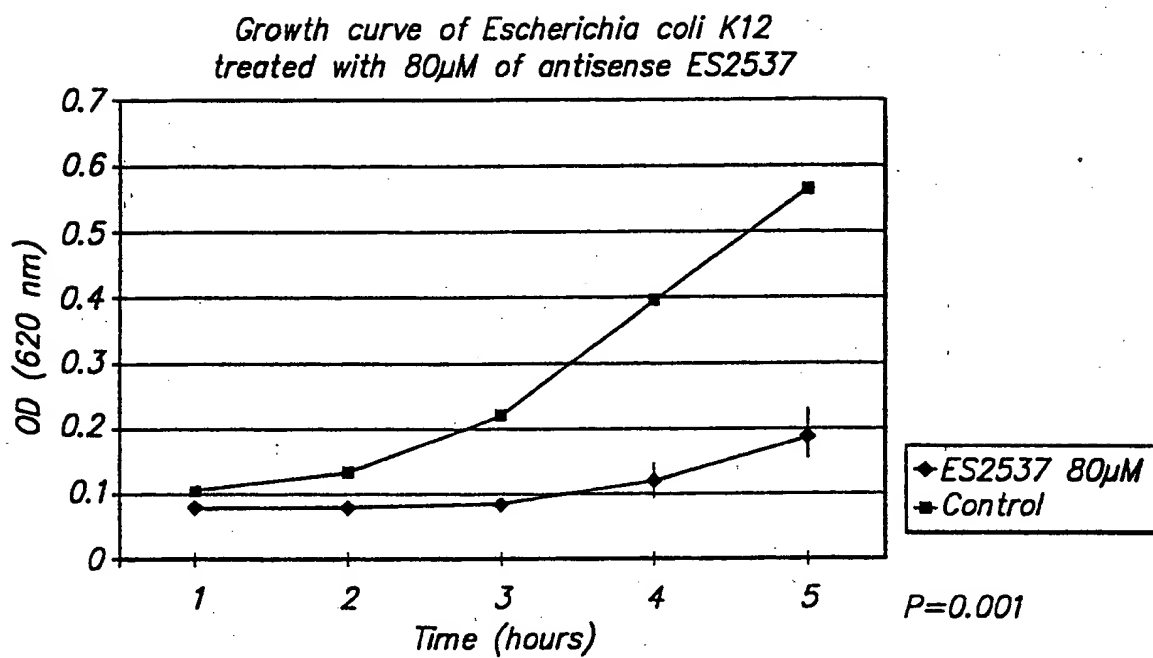
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**FIG. 19E**

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**FIG. 19F**

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**FIG. 19G**



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Filed on 10 July 1997 (10.07.97)

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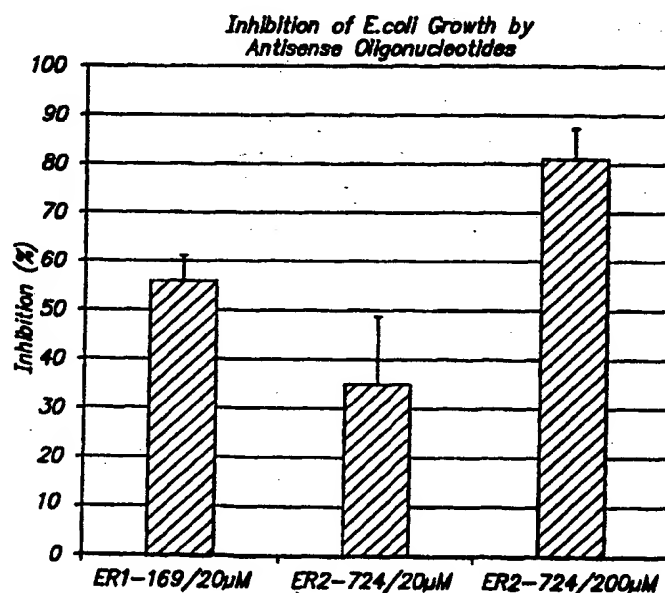
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(57) Abstract

The invention relates to antisense oligonucleotides which modulate the expression of the ribonucleotide reductase or the *secA* genes in microorganisms. This invention is also related to methods of using such oligonucleotides in inhibiting the growth of microorganisms. These antisense oligonucleotides are particularly useful in treating pathological conditions in mammals which are mediated by the growth of microorganisms.

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/CA 98/00666

A. CLASSIFICATION OF SUBJECT MATTER

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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	F.R. BLATTNER ET AL.: "The complete genome sequence of E. coli K-12" SCIENCE, vol. 277, 1997, pages 1453-1462, XP002089422 *see the whole article*	1-17
Y	WO 96 26276 A (THE GOVERNEMENT OF THE UNITED STATES OF AMERICA ET AL.) 29 August 1996 *SEE THE WHOLE PATENT*	1-17



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INTERNATIONAL SEARCH REPORT

International Application No

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Y	W.J. PHILIPP ET AL.: "An integrated map of the genome of the tubercle bacillus, M. tuberculosis H37 Rv, and comparison with M. leprae" PROCEEDINGS OF NATIONAL ACADEMY OF SCIENCES USA, vol. 93, 1996, pages 3132-3137, XP002089427 *see the whole article*	1-17
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INTERNATIONAL SEARCH REPORT

International Application No

PCT/CA 98/00666

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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PCT/CA 98/00666

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	M.I. ANAZODO ET AL.: "Characterization of GP12A, a potent inhibitor of HIV-1 gene expression and viral replication" NUCLEOSIDES AND NUCLEOTIDES, vol. 16, no. 7-9, 1997, pages 1241-1249, XP002089435 *see the whole article*	1-17
Y	M.I. ANAZODO ET AL.: "Sequence specific inhibition of gene expression by a novel antisense oligodeoxynucleotide phosphorothioate directed against a nonregulatory region of the HIV-1 genome" JOURNAL OF VIROLOGY, vol. 69, no. 3, 1995, pages 1794-1801, XP002089436 *see the whole article*	1-17
Y	R.H. BARKER ET AL.: "Inhibition of P. falciparum malaria using antisense oligodeoxynucleotides" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES USA, vol. 93, 1996, pages 514-518, XP002053162 *see the whole article*	1-17
Y	M.I. ANAZODO ET AL.: "Relative levels of inhibition of p24 gene expression by different 20-mer antisense oligonucleotide sequences targeting nucleotides +1129 to +1268 of the HIV1 gag genome: an analysis of mechanism" BIOCHEMICAL BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 229, 1996, pages 305-309, XP002089438 *see the whole article*	1-17
Y	M. SPEARMAN ET AL.: "Antisense oligodeoxyribonucleotide inhibition of TGF-beta1 gene expression and alterations in the growth and malignant properties of mouse fibrosarcoma cells" GENE, vol. 149, 1994, pages 25-29, XP002089439 *see the whole article*	1-17
Y	WO 94 12633 A (STIEFEL LABORATORIES, INC.) 9 June 1994 *see the whole patent*	1-17
Y	WO 94 08004 A (HYBRIDON, INC.) 14 April 1994 *see the whole patent*	1-17

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